OLYMPUS®

User's Manual

FLUOVIEW FV1000

CONFOCAL LASER SCANNING BIOLOGICAL MICROSCOPE

[OPERATION] FV10-SW Ver 5.0c

Petition

This user's manual is for the software to be run on Olympus FLUOVIEW FV1000 Confocal Laser Scanning Biological Microscope. To ensure safety, obtain optimum performance and familiarize yourself fully with this product, we recommend that you study this manual thoroughly before operation. This user's manual is composed of two volumes including "OPERATION INSTRUCTIONS" and "MAINTENANCE". Together with this manual, please also read the "SAFETY GUIDE", "HARDWARE GUIDE" of User's manual FLUOVIEW FV1000 and the instruction manual of the microscope in order to understand overall operation methods. To ensure the safety operation of laser system, we recommend you to study the manual of each laser and the light source equipment besides this manual.
Retain this manual in an easily accessible place near a system for future reference.

AX7274

CAUTION

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FLUOVIEW MANUAL CONFIGURATION

The FLUOVIEW system uses two manuals including this “User’s Manual” and the on-screen manual built into the software (“Online Help”).

The User’s Manual is composed of the five following volumes and subject matter:

OPERATION INSTRUCTIONS

Describes the operation procedures of the FLUOVIEW system, for example, methods for image acquisition and various image processing.

1 Getting Started FLUOVIEW 1-1
2 APPLIED OPERATIONS 2-1
Appendix A List of Hot Keys A-1
Appendix B Glossary B-1
Appendix C USER REGISTRATION OF FV1000 ..... C-1
Appendix D Change of Default Folder for [File I/O] Panel D-1
Appendix E List of Functions in the [Active Overlays] Dialog Box E-1
Appendix F Hand Switch and Microscope Frame Function Allocation F-1

MAINTENANCE

Describes maintenance of the FLUOVIEW system.

1 Software Setup 1-1
2 Maintenance of Major System Units 2-1
3 Setting the Confocal Aperture 3-1

TROUBLESHOOTING

Describes countermeasures in case trouble occurs.

1 TROUBLESHOOTING GUIDE 1-1

For Online Help, please see “1-3 Online Help” in “OPERATION INSTRUCTIONS” of this manual.

NOTATIONS IN THIS MANUAL

This manual complies with the following notations.

✧ Notation of Caution, Notes and Tips

Notation	Description
	Caution to prevent injuries to the user or damage to the product (including surrounding objects).
	Note for the user.
	Hint or one-point advice for user reference.

✧ Notation of panel, Command Buttons and Dialog Boxes

Notation	Description
[Acquire] panel	The name of a panel, dialog box, list box or check box is enclosed inside square brackets ([ ]).
<OK> button <Open File> button	The name of a button in a panel or dialog box is enclosed inside angled brackets (< >).

✧ Notation of Mouse Operations

Notation	Description
clicking	Action of pressing, then immediately releasing the mouse button.
double-clicking	Action of clicking the mouse button twice in quick succession.
dragging	Action of moving the mouse while holding down the mouse button, then releasing the mouse button at the desired destination.

(Note) In this manual, clicking, double-clicking and dragging involves pressing the left button of the mouse, unless otherwise specified.

✧ Notation of key operations

Notation	Description
[Enter]	The name of a key is enclosed inside [ ].
[Alt] + [F1]	The positive sign (+) expresses the combination of more than one key operation. For example, [Alt] + [F1] refers to pressing the [F1] key while holding the [Alt] key down.
Direction keys	Generic names given to the [→], [←], [↑] and [↓] keys.

✧ Notation of system-specific terms

Notation	Description
XY observation	Refers to observation with XY scanning.
(Other observations)	(The same principle also applies to other observations such as XZ, Xt, XYZ, XYt and XYZt.)

Note that some of the panels and dialog boxes shown in this manual are not the precise reproductions of the originals. Some windows are resized to facilitate the reading and some grayed-out characters are printed in readable characters.

Software Functional Configuration

This software uses panel-type windows.

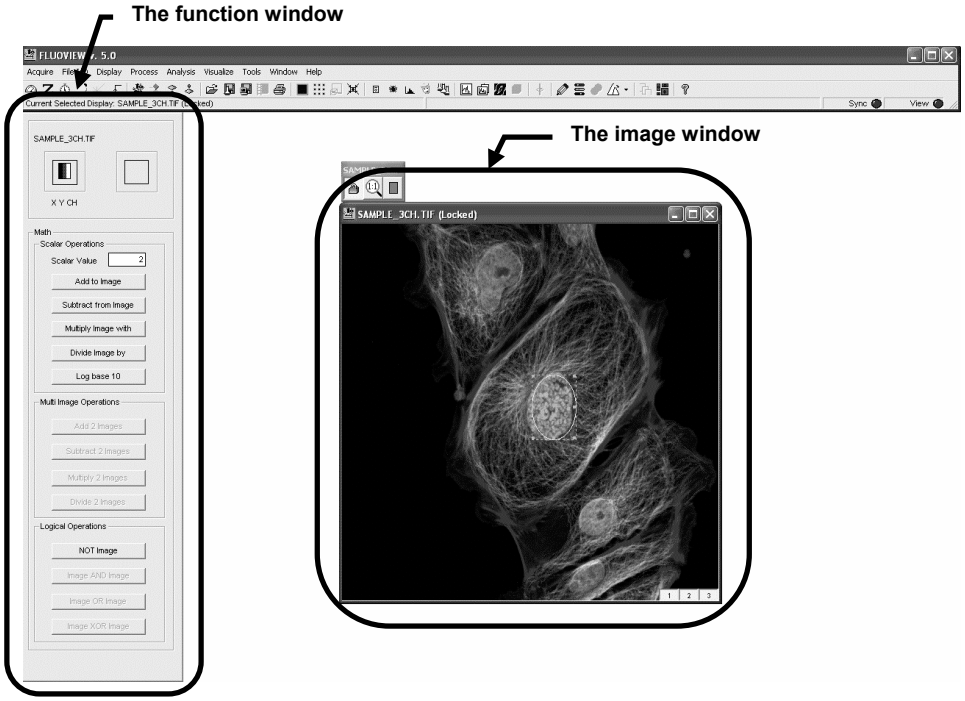
Usually, it is required to “select a menu then select the command to be executed” in order to execute a function provided by software. With the panel system, a software function can be executed easily by “selecting the panel page tab of the function to be executed”, just like when using a system notebook or file folder.

Function Window and Image Window

The FLUOVIEW software is organized by two kinds of windows, the function windows and the image .window.

The function windows include the [Acquire], [File I/O], [Tile], [Process], [Analyze] and [Visualize] panels.

The image .window shows either the [Live] panel or the panel image loaded from a file ([(filename)] window).



NOTE

In this manual, the function windows are referred to simply using their page tabs.

Namely, the [File I/O] panel of function windows is referred to simply as the [File I/O] panel.

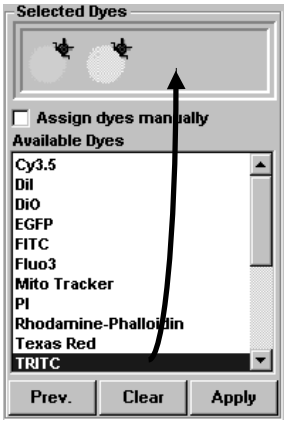
Panel Structure of the Software

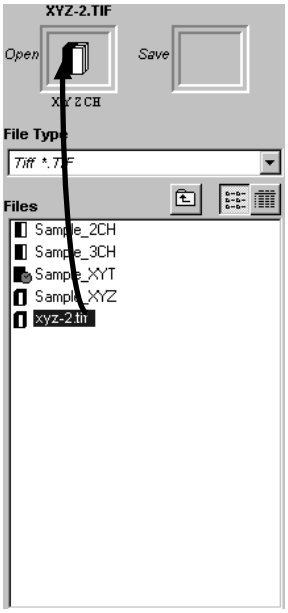
This software cannot show the all function panels at a glance.Please use the following list of the panels for reference in scrolling.

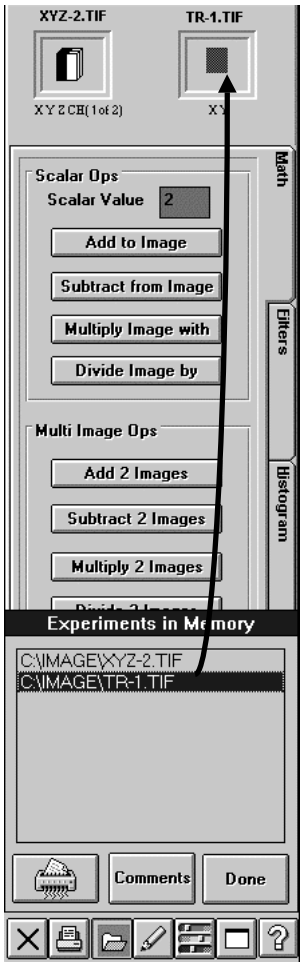
- Acquire — Sets up the image acquisition and executes actual acquisition.
  - Settings — Sets the zooming ratio and observation mode for image acquisition. Sets the microscope light path for image acquisition.
  - Z Stage — Sets the Z-direction scanning range for image acquisition.
  - Time Series — Sets the interval period for image acquisition.
  - Dyes — Sets the fluorescence dye method for image acquisition and reagent for each channel.
  - Lasers — Sets the intensity of laser and other option for image acquisition.
- File I/O — Saves, loads and deletes images.
- Tile — Changes the image display method.
- Process — Processes the acquired images.
  - Math — Performs mathematical and logical operations between images.
  - Filters — Filters images.
  - Histogram — Changes the image contrast.
  - Experiment Editor — Appends two images, adds and extracts the channel.
- Analyze — Analyzes image data.
  - Single — Obtains the intensity values, intensity distribution, length, area and average intensity in images.
  - Series — Obtains the change in the sum of intensity values in images.
- Visualize — Constructs an image from a different viewpoint or displays a 3D image.
  - Orientation — Sets the image rotation angle, direction and number.
  - Other Options — Sets the various 3D Rendering.

Icons Executed by Dragging & Dropping

This software selects image files and observation methods (dye name) by means of dragging & dropping. This allows simple selection based on an intuitive operation of "selecting an icon (image file or observation method), dragging it to the desired position and dropping it there".







Identification of Images Depending on the Observation Methods

Image Icon	Significance
	XZ observation
	XZ observation, 2-channel mode
	Xt observation
	Xt observation, 2-channel mode
	XZT observation
	XZT observation, 2-channel mode
	XY observation
	XY observation, 2-channel mode
	XYt observation
	XYt observation, 2-channel mode
	XYZ observation
	XYZ observation, 2-channel mode
	XYZt observation
	XYZt observation, 2-channel mode
	Point Scan
	Animation image
	Stereo 3D image: Image to be viewed with color eyeglasses.
	3 or more channels

On many occasions, FLUOVIEW displays image icons to allow identification of the observation method used when each image is acquired. (See table on the left.)

When the [File I/O], [Tile], [Process], [Analyze] or [Visualize] panel is selected, the icon of the image selected in the image window is displayed in a frame at the top of the function panel. The image icons are also displayed in the [Icon] field in the [Files] list box in the [File I/O] panel or during dragging of an image file.

Use these icons to identify the observation methods used in image acquisition.

TIP In all observation modes, the icons for 3 or more channels are identical.

OPERATION INSTRUCTIONS

On This Volume

This volume describes the operating procedures of the FLUOVIEW FV1000 system.
“Getting Started FLUOVIEW” contains information on the basic operation flow until acquisition of XY images.
“APPLIED OPERATIONS” provides detailed operating procedures of the system.
Please read this volume so that you can understand the system before use.

1 Getting Started FLUOVIEW 1-1

1-1 Basic Operations 1-1
1-1-1 Microscope 1-1
1-1-2 General Mouse Operation Procedures 1-7
1-1-3 Names of Major Panel and Window Controls and Their Functions 1-8
1-2 Outline of LSM Observation Procedures 1-9
1-2-1 Turning Power On 1-11
1-2-2 Focusing on the Specimen 1-12
1-2-2-1 Combination with BX 1-12
1-2-2-2 Combination with IX81 FVF 1-14
1-2-3 Setting the LSM Light Path 1-16
1-2-3-1 Combination with Upright Microscope (BX) 1-16
1-2-3-2 Combination with Inverted Microscope (IX81 FVF) 1-18
1-2-4 Selecting the Dyeing Method 1-20
1-2-5 Selecting the Filters 1-23
1-2-6 Setting the ND Filters 1-26
1-2-7 Setting the Observation Condition 1-27
1-2-7-1 Setting the Objective Magnification 1-27
1-2-7-2 Setting the Zoom Ratio to 1X 1-28
1-2-7-3 Setting the Channels 1-28
1-2-7-4 Setting the Highest Scan Speed 1-29
1-2-7-5 Setting the XY Observation Mode 1-30
1-2-7-6 Repeated Scanning Operation 1-30
1-2-7-7 Setting the Cross-section to be Observed 1-31
1-2-7-8 Setting the Area to be Observed 1-32
1-2-7-9 Setting a Lower Scan Speed 1-32
1-2-7-10 Stopping Repeated Scanning 1-33
1-2-8 Acquiring Image 1-33
1-2-9 Saving Image 1-34
1-2-10 Exiting from the Software, Turning Power Off 1-35

CONTENTS

1-3 Online Help 1-36
1-3-1 Function Help 1-36
1-3-2 Microscope Help 1-37
1-3-2-1 Configuring the Microscope 1-40
1-3-2-2 Parfocality Correction and Jog Sensitivity Adjustment 1-46
1-3-2-3 Configuring the Filters (When using a filter system) 1-52
1-3-2-4 Configuring the filters (When using a spectral detecting system) 1-56
1-3-2-5 Setting the C.A. Diameters 1-59

2 APPLIED OPERATIONS 2-1

2-1 General Operation Procedure 2-1
2-1-1 Image Acquisition Procedure (Section (A)) 2-3
2-1-2 Image Acquisition Procedure in an Observation Mode (Section (B)) 2-4
2-1-3 Examples of Operation Procedures 2-5
2-2 Image Acquisition 2-7
2-2-1 Image Acquisition in XY Observation Mode 2-8
2-2-1-1 Configuring the Microscope 2-9
2-2-1-2 Setting the Filters 2-15
2-2-1-3 Setting the ND Filters 2-17
2-2-1-4 Setting the Observation Condition 2-19
2-2-1-5 Acquiring Image 2-28
2-2-1-6 Acquiring Image in Accumulation Mode 2-29
2-2-1-7 Saving the Acquired Image in File 2-33
2-2-2 Image Acquisition in Other Observation Modes 2-34
2-2-2-1 XZ Observation Mode 2-34
2-2-2-2 XT Observation Mode 2-38
2-2-2-3 XZT Observation Mode 2-40
2-2-2-4 XYZ Observation Mode 2-46
2-2-2-5 XYT Observation Mode 2-51
2-2-2-6 XYZT Observation Mode 2-55
2-2-3 Differences in Image Acquisition Method Between Fluorescent and Transmitted

Images 2-61
2-2-3-1 Monochrome Image 2-61
2-2-3-2 Dual-Fluorochrome Image 2-64
2-2-3-3 Transmitted Image 2-67
2-2-4 Image Acquisition by Rotating It (Rotation Scan) 2-71
2-2-5 Image Acquisition of Only the Rectangular Position (Clip Scan) 2-72
2-2-6 Image Acquisition by Magnifying the Rectangular Position (Zoom-In Scan) 2-75
2-2-7 High-Speed Image Acquisition 2-78
2-2-8 Image acquisition to prevent crosstalk between fluorescence (Sequential Scan) 2-79
2-2-8-1 Virtual Channel Function 2-82
2-2-9 Image Acquisition of a Line at Desired Angle 2-88
2-2-10 Display the change of image intensity (Point Scan) 2-89
2-2-11 Image Acquisition on Desired Line (XZ, XT or XZT Observation) 2-94
2-2-12 Image Acquisition in the Laser Excitation Mode 2-97
2-2-12-1 Making REX Mask File 2-98
2-2-12-2 Example of FRAP experiment 2-103
2-2-13 Notes for image acquisition 2-112
2-2-13-1 Memory of setting information for scanning region 2-112
2-3 Saving, Opening and Shredding Images 2-113
2-3-1 Saving Images 2-116
2-3-1-1 Saving Images As a Series 2-116
2-3-1-2 Saving a Display 2-118
2-3-1-3 Saving Specified Area of Image 2-120
2-3-1-4 Saving Animation Images 2-123
2-3-1-5 File Types Available for Save 2-125
2-3-2 Opening Previously Saved Images 2-132
2-3-3 Shredding Images 2-133
2-3-4 Saving Comment Together with Image 2-135
2-3-5 Checking the Image Information/Acquisition Parameters 2-138
2-3-6 Saving the Image Information/Observation Condition 2-142
2-3-7 Saving/Reading the Region File 2-145

CONTENTS

2-3-7-1 Saving the Region File 2-145
2-3-7-2 Reading the Region File 2-147
2-4 Protocol processor 2-150
2-4-1 Starting the Protocol Processor 2-151
2-4-2 Editing the Protocol 2-152
2-4-2-1 Description of Setting Items 2-153
2-4-2-2 Command List 2-160
2-4-2-3 Protocol Repetition Processing 2-165
2-4-2-4 Input supporting function 2-167
2-4-2-5 Protocol procedure supporting function 2-169
2-4-3 Saving the Protocol 2-170
2-4-4 Executing the Protocol 2-172
2-4-5 Loading a Protocol 2-174
2-4-6 Loading a protocol of previous format 2-175
2-4-7 COM Communication function 2-177
2-4-8 Example of assembling a protocol 2-178
2-4-8-1 Example of Setting Procedure of FRAP Observation 2-178
2-4-8-2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing) 2-187
2-4-9 Pop-up Menu 2-195
2-4-10 Restrictions of [PAPP] setting 2-196
2-4-10-1 Restrictions with [Mode] and [Sub Mode] 2-196
2-5 Changing the Image Display Method 2-198
2-5-1 Displaying an Image in Simulated Colors 2-198
2-5-2 Editing the LUT (Look Up Table) 2-200
2-5-2-1 LUT Graph Editing According to Colors 2-200
2-5-2-2 LUT Graph Editing by Gamma Correction 2-202
2-5-3 Switching the Displayed Channels (Ch1 – Ch5) 2-203
2-5-4 Displaying Images of Multiple Channels Simultaneously (Side By Side Views, Over And Under Views, Single View) 2-204
2-5-4-1 Displaying Images Separately Per Channel (Side By Side Views, Over And Under Views) 2-205

2-5-4-2 Displaying Merged Image of Multiple Channels (Single View).....2-206
2-5-5 Changing the Number of Divided Images 2-208
2-5-5-1 Increasing the Number of Divided Images 2-208
2-5-5-2 Decreasing the Number of Divided Images.....2-210
2-5-6 Switching the Display Method of Multiple Images 2-211
2-5-7 Displaying Multiple Image Slices Together 2-213
2-5-7-1 Displaying Multiple Images Per Channel.....2-214
2-5-7-2 Displaying Images of Two Channels Together.....2-216
2-5-7-3 Displaying Time-Lapse Images 2-217
2-5-7-4 Displaying Multiple Multiple sections Images 2-218
2-5-7-5 Displaying Same Images in Different Display Methods.....2-218
2-5-7-6 Re-arranging Images Using the Same Display Method 2-221
2-5-7-7 Displaying Different Images Together 2-221
2-5-8 Magnifying/Reducing an Image.....2-224
2-6 Image Processing 2-225
2-6-1 Filtering.....2-225
2-6-1-1 Contour Enhancement.....2-226
2-6-1-2 Noise Reduction 2-229
2-6-1-3 Image Sharpening 2-230
2-6-1-4 DIC Correcting DIC Level Irregularities 2-231
2-6-2 Contrast Conversion.....2-233
2-6-3 Mathematical Operations Between Images.....2-236
2-6-3-1 Image Addition.....2-236
2-6-3-2 Image Subtraction.....2-239
2-6-3-3 Image Multiplication 2-239
2-6-3-4 Image Division 2-240
2-6-3-5 NOT Image 2-241
2-6-3-6 Image AND Image 2-243
2-6-3-7 Image OR Image 2-244
2-6-3-8 Image XOR Image 2-244
2-6-4 Brightness overlap level between 2 channels (Colocalization).....2-246
2-6-4-1 Annotation Mode.....2-247

CONTENTS

2-6-4-2 Colocalization for series image data set 2-254
2-6-4-3 Image measurement 2-255
2-6-5 Appending image (Append) 2-258
2-6-5-1 Appending two images 2-258
2-6-5-2 Appending image from several image data set 2-262
2-6-6 Extract image (Crop) 2-267
2-7 Image Analysis 2-274
2-7-1 Checking the Intensity of a Specific Part 2-275
2-7-1-1 Intensity Values on a Line (Line Profile) 2-275
2-7-1-2 Intensity Values on a Planar Region (Bird's Eye View) 2-278
2-7-2 Checking the Intensity Distribution of a Specific Part 2-283
2-7-2-1 Intensity Distribution on a Line (Histogram) 2-283
2-7-2-2 Intensity Distribution on a Planar Region (Histogram) 2-284
2-7-3 Image Measurement 2-286
2-7-3-1 Length Measurement 2-286
2-7-3-2 Area Measurement 2-286
2-7-3-3 Measuring the Change in Mean Value of Intensity 2-287
2-7-3-4 Measuring the Change in Integrated Intensity 2-293
2-8 Building an Image from a Different Viewpoint 2-295
2-8-1 Building Extended Focus Image from XYZ Image 2-295
2-8-1-1 Display Switching to Built Image 2-295
2-8-1-2 Turning Built Image into Single Image 2-298
2-8-1-3 Turning Built Image into time series image 2-300
2-8-2 Building line images to be viewed in Z direction 2-301
2-9 Viewing 3D Image 2-305
2-9-1 Successive Display of Images 2-307
2-9-1-1 Changing the Successive Display Speed 2-308
2-9-1-2 Changing the successive image display position 2-309
2-9-2 Animation 2-310
2-9-3 Building Stereo 3D Images 2-312
2-9-4 Building a 3D Image to be Viewed Through Color (Red/Green) Eyeglasses 2-314
2-10 Viewing Images Following the Progress of Time 2-316

2-10-1 Displaying Images Together 2-316
2-10-2 Displaying Images Successively 2-316
2-11 Transferring Data to Another Application 2-319
2-11-1 Transferring Analysis Data to Another Application 2-319
2-11-2 Transferring the Plot Image of Analysis Data to Another Application 2-322
2-11-3 Transferring Image Data to Another Application (Paint, etc.) 2-323
2-12 Entering Comment in Image 2-324
2-12-1 Writing Characters in Image 2-324
2-12-2 Displaying the Image Intensity 2-326
2-12-3 Displaying the X-coordinate/Y-coordinate of the Image 2-327
2-12-4 Drawing a Figure in Image 2-328
2-12-5 Drawing a Scale in Image 2-329
2-12-6 Drawing an Arrow in Image 2-330
2-12-7 Drawing Color Bars in Image 2-331
2-12-8 Deleting Comment 2-332
2-12-9 Moving Comment 2-333
2-12-10 Changing the Comment Size 2-334
2-12-11 Changing the Comment Color 2-335
2-12-12 Changing the Comment Font 2-336
2-13 Image Output at Printer 2-337
2-14 Merger/Extraction of Image Channels 2-338
2-14-1 Setting the Range of Multiple Image Slices 2-338
2-14-2 Merging Image Channels 2-339
2-14-3 Extracting Channels from Image 2-344
2-15 Changing the Chart Display Method 2-348
2-15-1 [Chart] Panel 2-348
2-15-2 [Series] Panel 2-356
2-16 Pop-up Menus 2-359

Appendix A List of Hot Keys A-1

CONTENTS

Appendix B Glossary B-1

Appendix C USER REGISTRATION OF FV1000 C-1

Appendix C-1 User Registration..... C-1
Appendix C-2 Logging into the FV1000..... C-4
Appendix C-3 Deleting a User C-5

Appendix D Change of Default Folder for [File I/O] Panel D-1

Appendix E List of Functions in the [Active Overlays] Dialog Box E-1

Appendix E-1 Coordinate Position Data.....E-1
Appendix E-1-1 X-Coordinate E-1
Appendix E-1-2 Y-Coordinate E-2
Appendix E-1-3 Other E-3
1 Z Position E-3
2 T Position E-3
3 Animation E-3
Appendix E-2 Intensity Data E-4
Appendix E-3 Other E-5
Appendix E-3-1 Channel Number E-5
Appendix E-3-2 Objective Power E-5
Appendix E-3-3 Date of Image Capturing E-5
Appendix E-3-4 Time of Image Capturing..... E-5
Appendix E-3-5 Image File Name..... E-5

Appendix F Hand Switch and Microscope Frame Function

Allocation F-1

Appendix F-1 Hand Switch Functions F-1
Appendix F-1-1 BX/BXWI F-1
Appendix F-1-2 IX.....F-2
Appendix F-2 Microscope Frame Functions F-3
Appendix F-2-1 BX F-3
Appendix F-2-2 IX.....F-4
Appendix F-2-3 Focus Adjustment Knob F-5
1 BX..... F-5
2 IX F-5

1 Getting Started FLUOVIEW

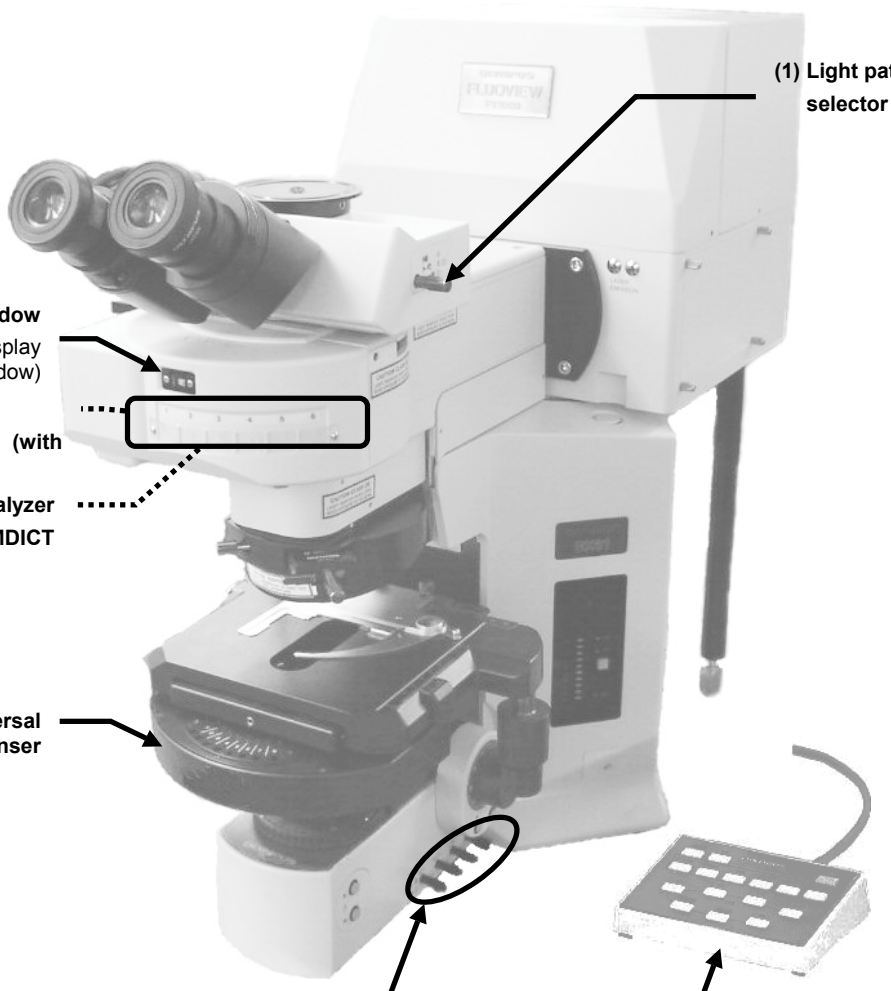
1-1 Basic Operations

1-1-1 Microscope

The following figure shows the major controls of a microscope. The actual configuration of the modules including the specimen stage, revolving nosepiece and lighting equipment may differ from those shown below.

For detailed microscope operation procedures, refer to the instruction manuals of your microscope.

Combination with BX61



(This figure shows the case of BX)

(1) Light path selector knob

Select the light path between the visual observation and photography observation.

- See the following table and set the knob to the position corresponding to the required light path.

Light path selector knob	Symbol	Intensity Ratio
Pushed in		100% for the visual observation
Middle position		20% for the visual observation, 80% for photography observation
Pulled out		100% for photography observation

(2) Cube turret

Select the fluorescence observation tube by rotating the turret.

- Engage the desired cube in the light path for visual fluorescence observation or visual transmitted light observation.
- For laser microscopy, rotate the turret to page tab . (Set cube turret in the state that mirror cube is entered.)

(3) Analyzer IX2-MDICT

Polarizing plate for use in differential interference observation and polarized light observation.

- Rotate the turret to engage the IX2-MDICT in the light path for visual transmitted light differential interference observation or transmitted polarized light observation.

(4) Transmitted light DIC slider U-DICTS/WI-DICTHRA (built-in FV10-SRE or FV10-SNPXLU of BX61WI)

This is the prism for use in differential interference observation.

- Engage the transmitted light DIC in the light path for laser differential interference observation or visual transmitted light differential interference observation.

  Leaving the transmitted light DIC engaged during laser fluorescence observation will degrade the resolution somewhat. We recommend disengaging the transmitted light DIC from the light path when simple laser fluorescence observation is required.

NOTE

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may degrade. (This applies to both visual observation and laser differential interference observation.)

(5) Filters

These filters are used to adjust the transmitted light.

- Be sure to disengage any filter from the light path for transmitted observation using lasers. Leaving a filter engaged in the light path will degrade the image quality.
  When you perform transmitted observation using laser with BX61WI, use the filter knob to disengage the LBD from the light path and engage the FR (Frost) into the light path. Disengaging the FR (Frost) from the light path may generate interference fringes on an image.

(6) Universal condenser

Condenser for transmitted lighting. In addition, the rotary turret for the transmitted light DIC prism and the polarizing plate for differential interference observation (polarizer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC prism matching the objective in use in the light path (For both visual observation and laser differential interference observation).
- To perform visual differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(7) Hand switch U-HSTR2 (U-FH is optionally available with BX61/BX61WI)

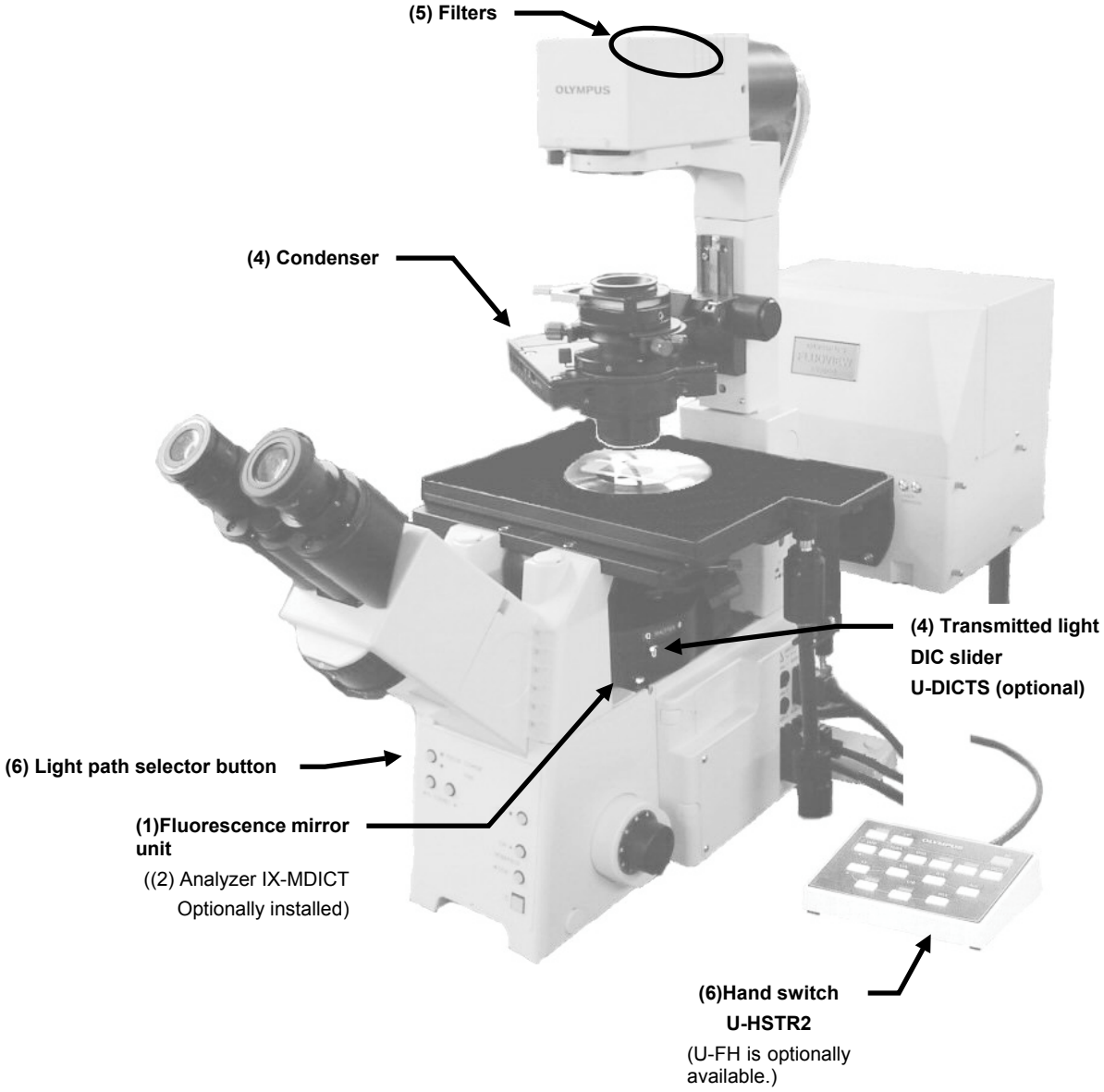
This is the hand switch to operate the BX motorized system.

NOTE

Connect the filter wheel of BX to connector of the following in the back of UCB.

- **FW0: FW1**
- **FWR: FW2**
- **FWT: FW3**

Combination with IX81 FVF



(1) Fluorescence mirror unit

Select the fluorescence observation tube by rotating the turret.

- Engage the desired cube in the light path for visual fluorescence observation.
- For laser microscopy, rotate the turret to page tab 1. (Set turret in the state that mirror cube is entered.)

(2) Analyzer IX2-MDICT

Polarizing plate for use in differential interference observation and polarized light observation.

- Rotate the cube turret to engage the IX2-MDICT analyzer into the light path for visual transmitted light differential interference observation or transmitted polarized light observation.

(3) Transmitted light DIC slider U-DICTS

This is a prism for use in differential interference observation.

- Engage U-DICTS in the light path for laser differential interference observation or visual transmitted light differential interference observation.
  Leaving U-DICTS engaged during laser fluorescence observation will degrade image quality somewhat. We recommend disengaging the U-DICTS from the light path when simple laser fluorescence observation is required.

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it inscribes the field of view. Otherwise the contrast may degrade. (This applies to both visual observation and laser differential interference observation.)

(4) Condenser, polarizing plate

Condenser for transmitted lighting.

In addition, the rotary turret for the transmitted light DIC and the polarizing plate for differential interference observation (analyzer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC (optional) matching the objective in use in the light path (For both visual observation and laser differential interference observation).
- To perform visual differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(5) Filters

These filters are used to adjust transmitted light.

- For transmitted observation using laser, disengage the LBD filter from the light path and engage the FR (frost) filter in the light path by operating the filter levers. If the FR filter is disengaged from the light path, the image may suffer from stripe interference.

(6) Light path selector button

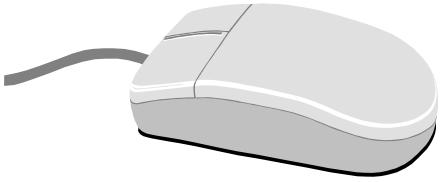
Select the light path between the visual observation and photography observation.

- When < > LED is lighted, visual observation can be done.
- When < > LED is lighted, TV or photography observation can be done.

(7) Hand switch (U-FH is optionally available.)

This is the hand switch to operate the IX motorized system.

1-1-2 General Mouse Operation Procedures



Use the mouse to select a command, character string or button. Use the left button of the mouse unless otherwise specified.

To select or execute something: Clicking

To click the mouse, place the mouse pointer on the desired function and press the mouse button once.

(Pressing the right button of the mouse is referred to as right-clicking.)

To select something and execute its function: Double clicking

To double-click, place the mouse pointer on the desired function and press the mouse button successively twice.

To move something: Dragging

To drag, place the mouse pointer on the desired function, and while pressing and holding the mouse button, move the mouse to the desired destination. At the desired destination, release the mouse button.

(Dragging by pressing the right button of the mouse is referred to as right-dragging.)

One Point!

When the mouse is moved, the picture of arrow on the screen moves accordingly. The picture which moves on the screen as the mouse is moved is referred to as the mouse pointer.

1-1-3 Names of Major Panel and Window Controls and Their Functions

The window as shown below is displayed when FLUOVIEW starts up. FLUOVIEW uses panel-type windows.

This section describes the names of the major controls displayed in panels and windows.

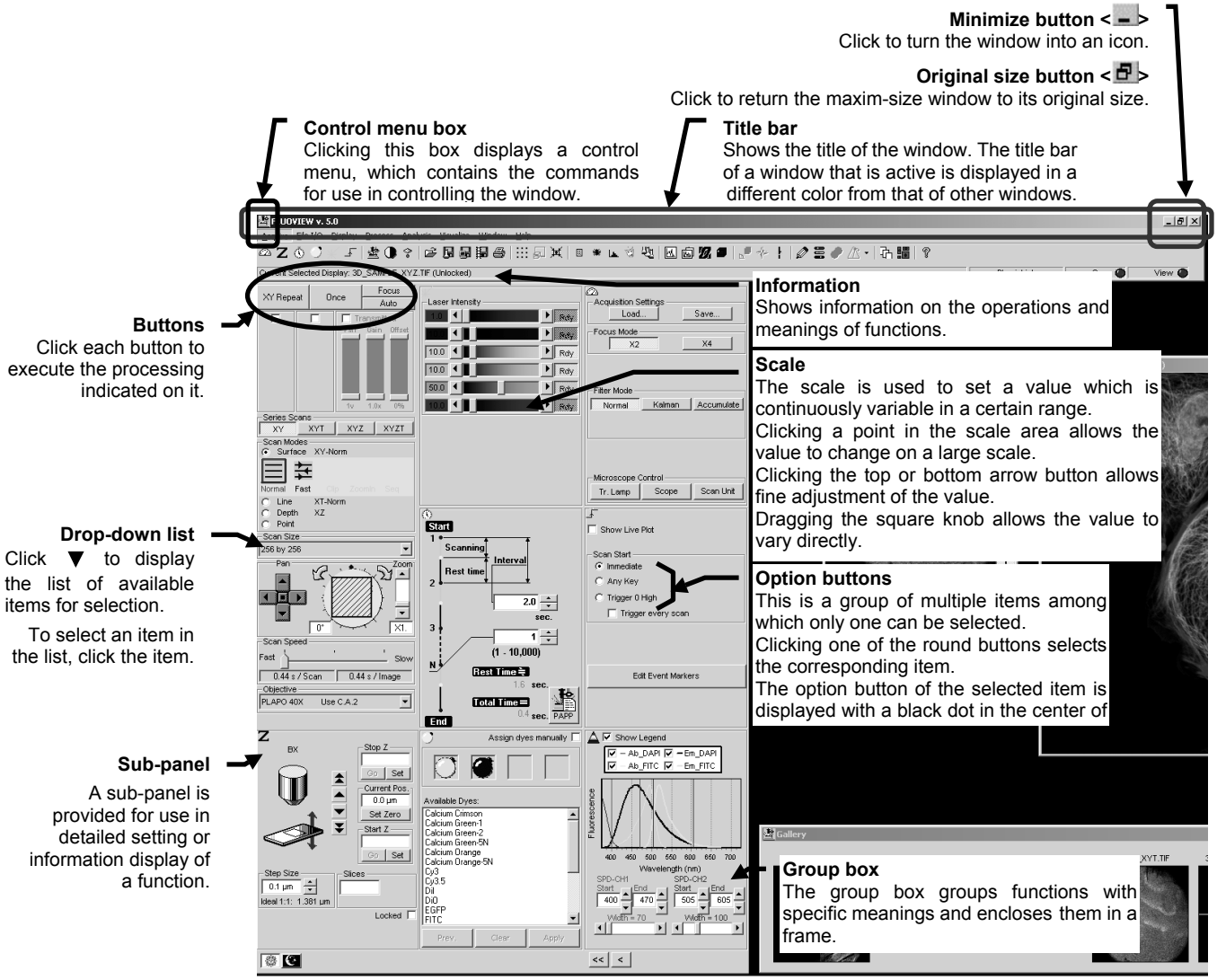
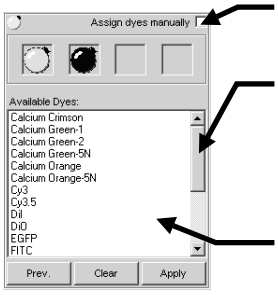

Minimize button <>
Click to turn the window into an icon.
Original size button <>
Click to return the maxim-size window to its original size.
Control menu box
Clicking this box displays a control menu, which contains the commands for use in controlling the window.
Title bar
Shows the title of the window. The title bar of a window that is active is displayed in a different color from that of other windows.
Information
Shows information on the operations and meanings of functions.
Buttons
Click each button to execute the processing indicated on it.
Scale
The scale is used to set a value which is continuously variable in a certain range.
Clicking a point in the scale area allows the value to change on a large scale.
Clicking the top or bottom arrow button allows fine adjustment of the value.
Dragging the square knob allows the value to vary directly.
Drop-down list
Click ▼ to display the list of available items for selection.
To select an item in the list, click the item.
Option buttons
This is a group of multiple items among which only one can be selected.
Clicking one of the round buttons selects the corresponding item.
The option button of the selected item is displayed with a black dot in the center of
Sub-panel
A sub-panel is provided for use in detailed setting or information display of a function.
Group box
The group box groups functions with specific meanings and encloses them in a frame.

Fig. 1-1 Window and Major Functions



Check box
Clicking this box enables or disables the indicated item. The item is enabled when the check box is checked (X).

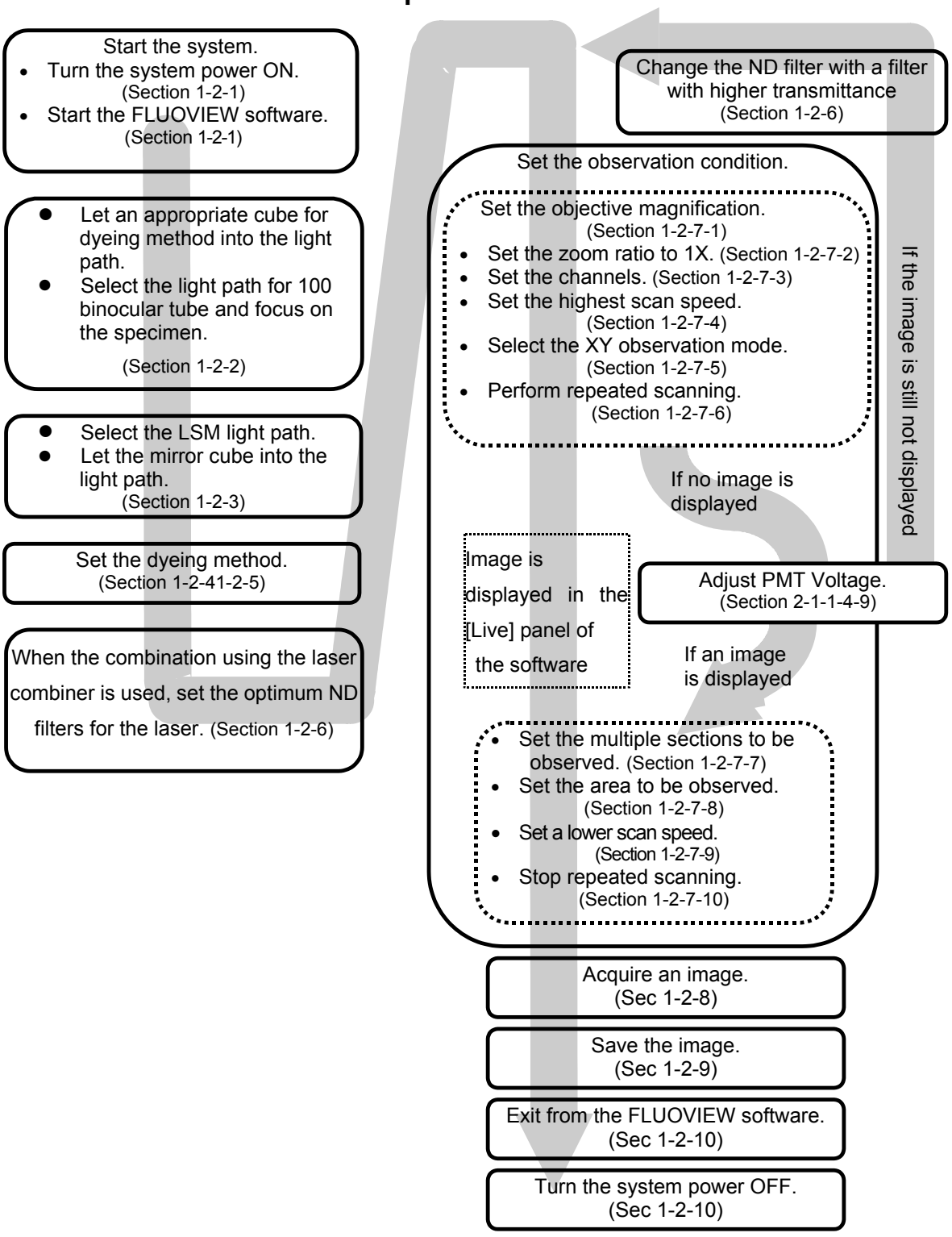
Scroll bar
The scroll bar is displayed when there are too many data items to be displayed in a field at once, and is used to display the data items outside the field. Clicking a point in the scroll area allows data items to be scrolled in large steps.
Clicking the top or bottom arrow button allows fine scrolling of the data items.
Dragging the square knob allows direct scrolling.

List box
Shows the list of available items for selection. All items in the list can be displayed by scrolling. To select an item in the list, double-click or drag the item.

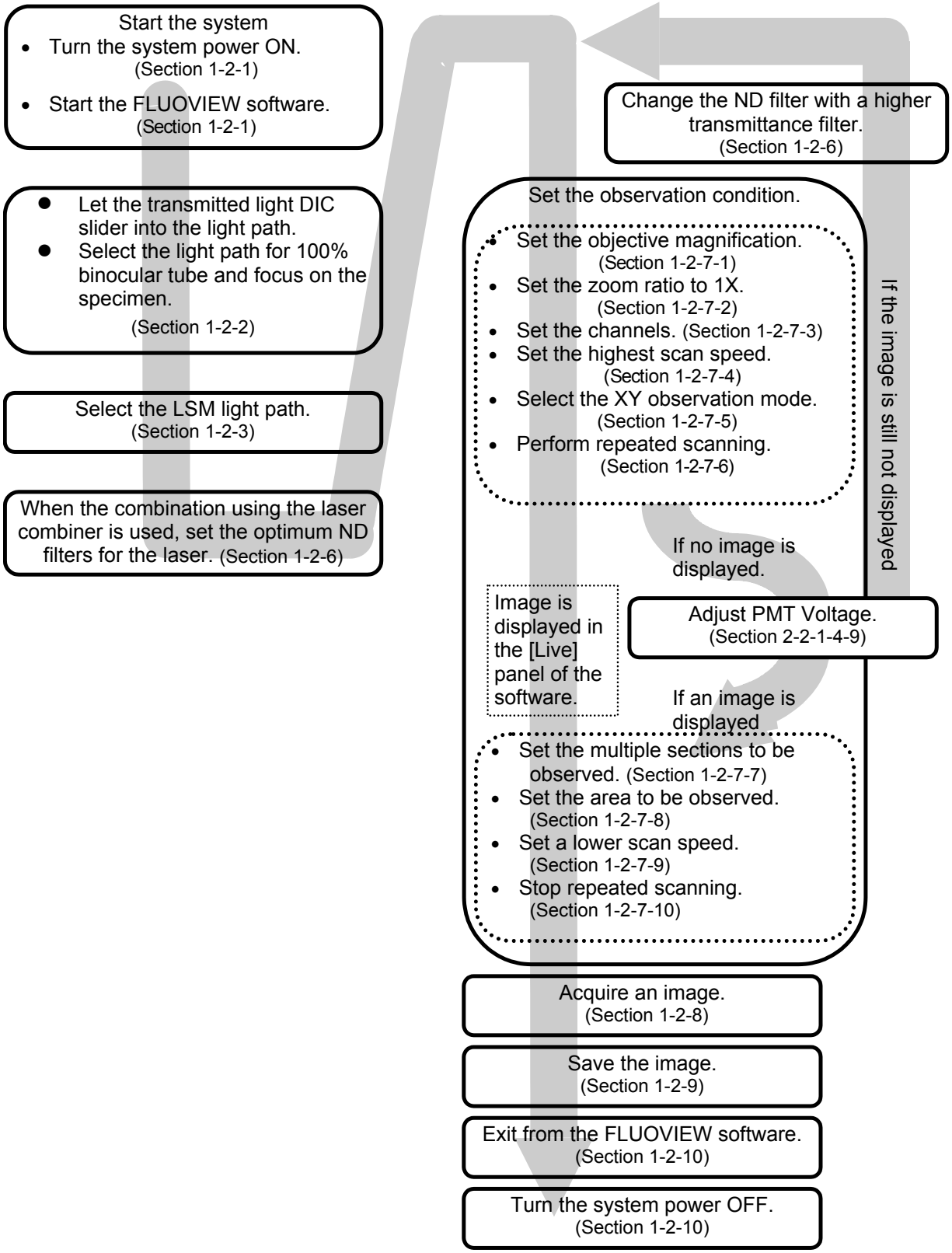
Fig. 1-2 Sub-panel and Major Functions

1-2 Outline of LSM Observation Procedures

- **Fluorescence observation procedure**


Start the system.
• Turn the system power ON. (Section 1-2-1)
• Start the FLUOVIEW software. (Section 1-2-1)
• Let an appropriate cube for dyeing method into the light path.
• Select the light path for 100 binocular tube and focus on the specimen.
(Section 1-2-2)
• Select the LSM light path.
• Let the mirror cube into the light path.
(Section 1-2-3)
Set the dyeing method. (Section 1-2-41-2-5)
When the combination using the laser combiner is used, set the optimum ND filters for the laser. (Section 1-2-6)
Change the ND filter with a filter with higher transmittance (Section 1-2-6)
Set the observation condition.
Set the objective magnification. (Section 1-2-7-1)
• Set the zoom ratio to 1X. (Section 1-2-7-2)
• Set the channels. (Section 1-2-7-3)
• Set the highest scan speed. (Section 1-2-7-4)
• Select the XY observation mode. (Section 1-2-7-5)
• Perform repeated scanning. (Section 1-2-7-6)
If the image is still not displayed
If no image is displayed
Image is displayed in the [Live] panel of the software
Adjust PMT Voltage. (Section 2-1-1-4-9)
If an image is displayed
• Set the multiple sections to be observed. (Section 1-2-7-7)
• Set the area to be observed. (Section 1-2-7-8)
• Set a lower scan speed. (Section 1-2-7-9)
• Stop repeated scanning. (Section 1-2-7-10)
Acquire an image. (Sec 1-2-8)
Save the image. (Sec 1-2-9)
Exit from the FLUOVIEW software. (Sec 1-2-10)
Turn the system power OFF. (Sec 1-2-10)

- **Transmitted light observation procedure**


Start the system
• Turn the system power ON. (Section 1-2-1)
• Start the FLUOVIEW software. (Section 1-2-1)
• Let the transmitted light DIC slider into the light path.
• Select the light path for 100% binocular tube and focus on the specimen. (Section 1-2-2)
Select the LSM light path. (Section 1-2-3)
When the combination using the laser combiner is used, set the optimum ND filters for the laser. (Section 1-2-6)
Change the ND filter with a higher transmittance filter. (Section 1-2-6)
Set the observation condition.
• Set the objective magnification. (Section 1-2-7-1)
• Set the zoom ratio to 1X. (Section 1-2-7-2)
• Set the channels. (Section 1-2-7-3)
• Set the highest scan speed. (Section 1-2-7-4)
• Select the XY observation mode. (Section 1-2-7-5)
• Perform repeated scanning. (Section 1-2-7-6)
If the image is still not displayed
If no image is displayed.
Image is displayed in the [Live] panel of the software.
Adjust PMT Voltage. (Section 2-2-1-4-9)
If an image is displayed
• Set the multiple sections to be observed. (Section 1-2-7-7)
• Set the area to be observed. (Section 1-2-7-8)
• Set a lower scan speed. (Section 1-2-7-9)
• Stop repeated scanning. (Section 1-2-7-10)
Acquire an image. (Section 1-2-8)
Save the image. (Section 1-2-9)
Exit from the FLUOVIEW software. (Section 1-2-10)
Turn the system power OFF. (Section 1-2-10)

1-2-1 Turning Power On

Set the power switch of each unit to ON, then start the software.

For details, see sections 1-1 “Turning the Power On” and section 1-2 “Starting the Software” in Volume II [PREPARATION For OPERATION] of [HARDWARE GUIDE] of the FV1000 User’s Manual.

1-2-2 Focusing on the Specimen

1-2-2-1 Combination with BX

1. Select the light path for 100% eyepiece by pushing in the light path selector knob (1) fully to the stop position.

2. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

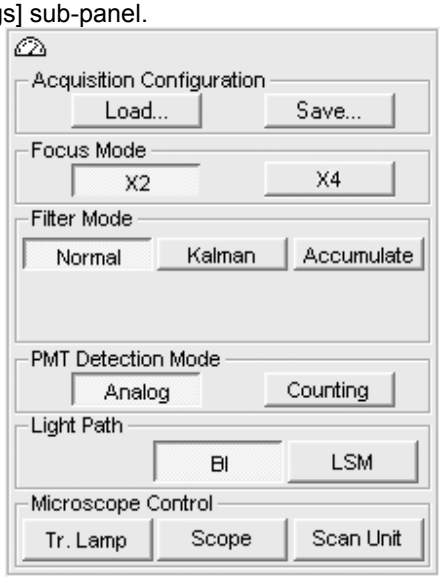
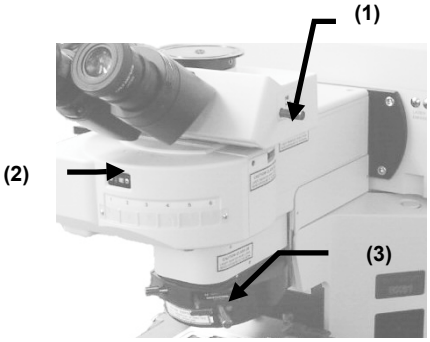
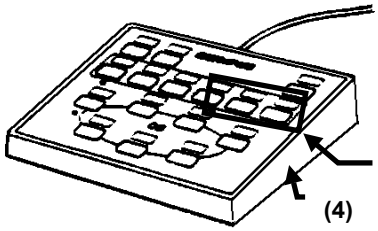


Fig. 1-3 [Settings] Sub-panel



3. Select the <BI> button in the [Light Path] group box.
   The <BI> button looks pushed in to indicate that it is selected.

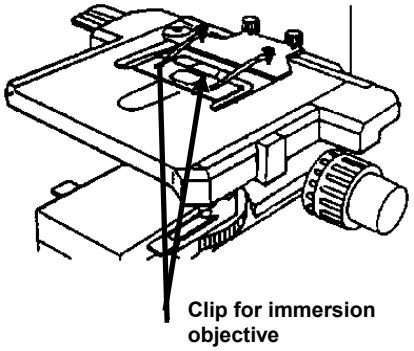
4. Push the cube button (5) of the hand switch (4) to engage the optimum cube for specimen dye. In the cube display window (2), the selected cube is displayed.



5. When you do the transmitted light observation, push the transmitted light DIC slider (3) and let it into the light path.

6. Focus on the specimen by looking into the eyepiece. Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the BX microscope.)

When focus is aligned with use of focus handle of microscope or U-FH, uncheck checkbox of [Locked] on [Z Stage] sub panel inside [Acquire] panel. (See 2-2-1-4-7 of this manual.) When [Locked] checkbox is checked, the handle cannot be operated.



Clip for immersion objective

NOTE

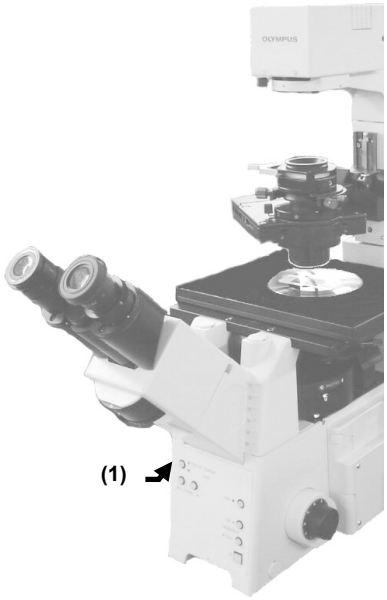
The specimen may float during oil-immersed observation. In this case, prepare an optional clip for immersion objective and attach it as shown on the left.

If you want to use a differential interference unit in transmitted light observation, refer to the instruction manual of your microscope.

NOTE

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may become degrade.

1-2-2-2 Combination with IX81 FVF



1. Push the light path selector button (1) on the front of the microscope to (when using Manual microscope).

   From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel, and Select the <BI> button in the [Light Path] group box (when using Motorized microscope).

   The <BI> button looks pushed in to indicate that it is selected.

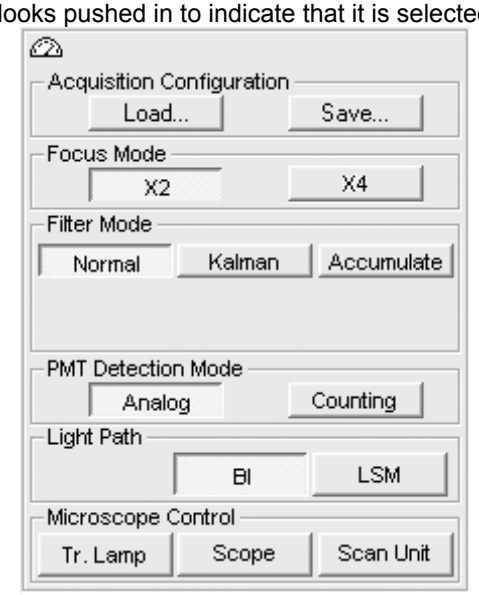
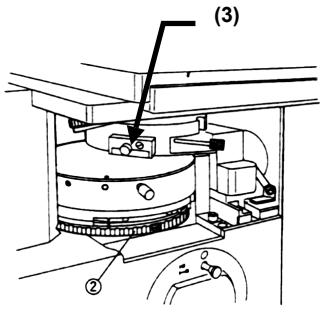


Fig. 1-4 [Settings] Sub-panel

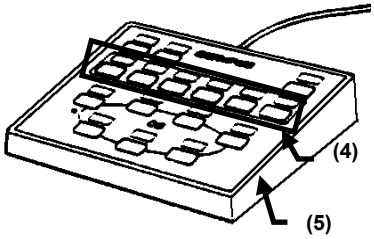


2. Engage the optimum cube for specimen dye by pressing the cube turret (2).

   (when using Manual microscope)

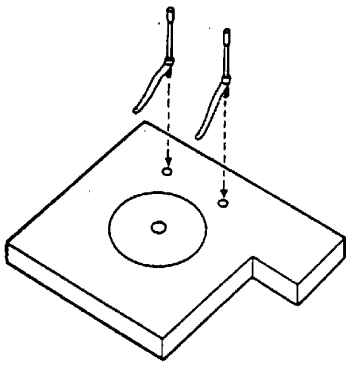
   Engage the optimum cube for specimen dye by operating the cube button (4) on the hand switch (5).(when using Motorized microscope)

3. When you do the transmitted light observation, push the transmitted light DIC slider (3) and let it into the light path.



4. Focus on the specimen by looking into the eyepiece. Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the IX71/81 microscope.)

When focus is aligned with use of focus handle of microscope or U-FH, uncheck checkbox of [Locked] on [Z Stage] sub panel inside [Acquire] panel. (See 2-2-1-4-7 of this manual.) When [Locked] checkbox is checked, the handle cannot be operated.



NOTE

The specimen may float during oil-immersed observation. In this case, prepare a stage clip (U-SCL) and attach it to the microscope as shown on the left.

1-2-3 Setting the LSM Light Path

1-2-3-1 Combination with Upright Microscope (BX)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

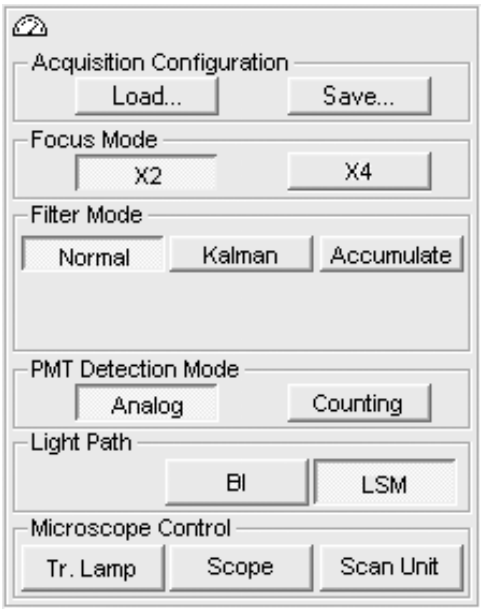


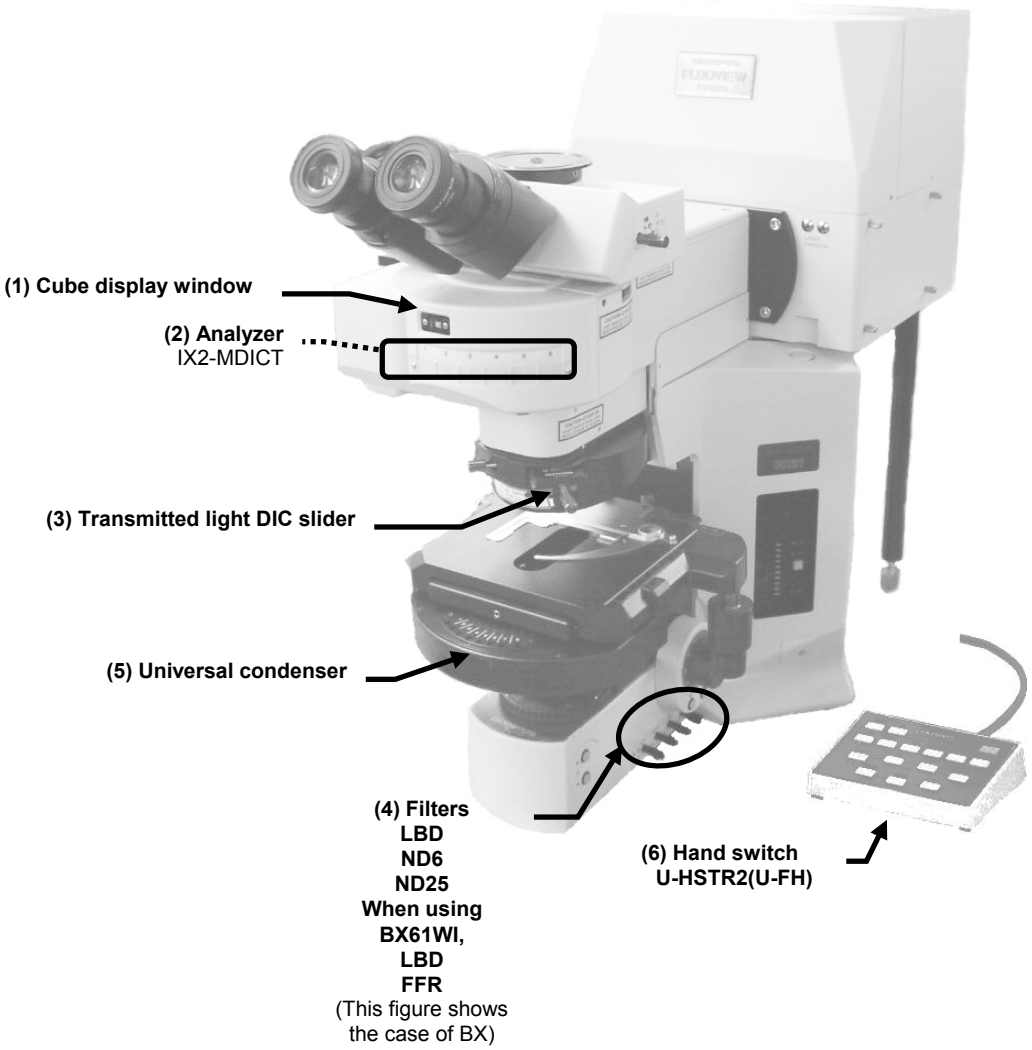
Fig. 1-5 [Settings] Sub-panel

2. Select the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)

3. Push the hand switch button to (8) set **1** to be displayed in the cube display window (1) on the reflected light fluorescence vertical illuminator

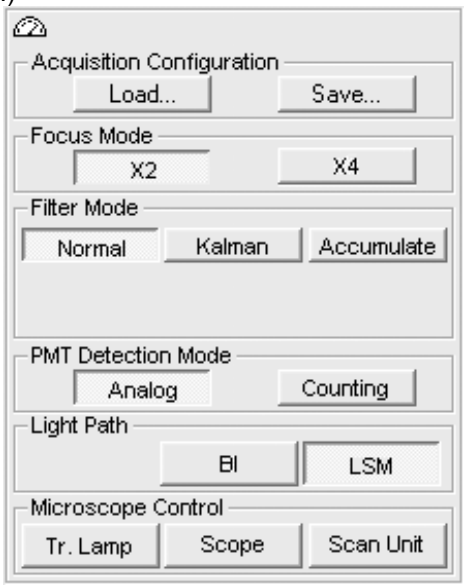
4. When only fluorescence observation is required, disengage the transmitted DIC slider (3) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the transmitted DIC slider and the optimum transmitted light DIC for the objective in the light path by operating the universal condenser (5).

5. For transmitted light observation, disengage any filter (4) from the light path.
When you perform transmitted observation using laser with BX61WI, use the filter knob to disengage the LBD from the light path and engage the FR (Frost) into the light path. Disengaging the FR (Frost) from the light path may generate interference fringes on an image.



1-2-3-2 Combination with Inverted Microscope (IX81 FVF)

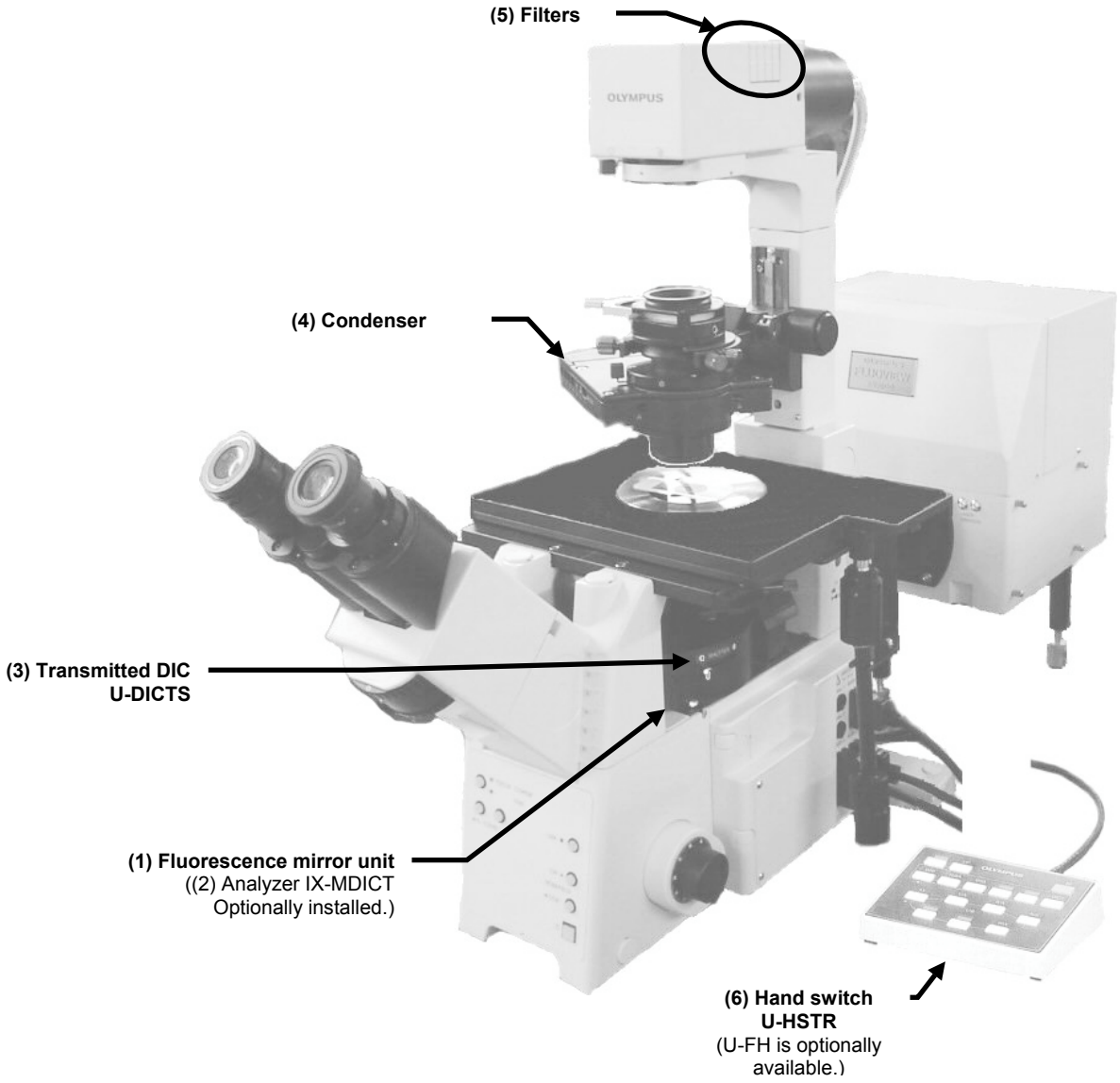
1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel, and .select the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)



2. Push the hand switch button (7) to set reflected light fluorescence mirror unit to **1**.

3. When a fluorescence observation alone is required, disengage the U-DICTS transmitted DIC slider (3) by setting the switch to the pulled-out position. When a transmitted light DIC observation or a simultaneous fluorescence & transmitted light DIC observation is required, engage the transmitted DIC slider (3) and the optimum transmitted DIC slider for the objective into the light path by operating the universal condenser (4).
   In a simultaneous fluorescence & transmitted light differential interference observation, leaving the transmitted DIC slider (3) within the light path degrades the fluorescence image resolution to some extent.

4. During the transmitted light observation, be sure to disengage the filter (5) from the light path.



1-2-4 Selecting the Dyeing Method

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

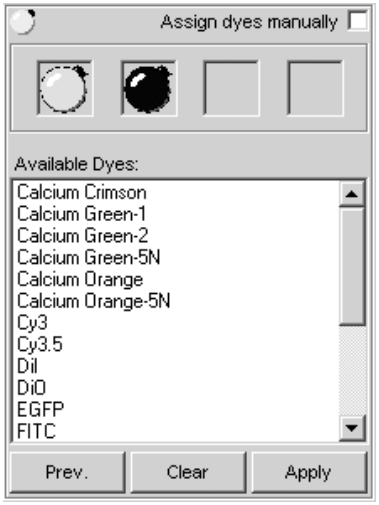
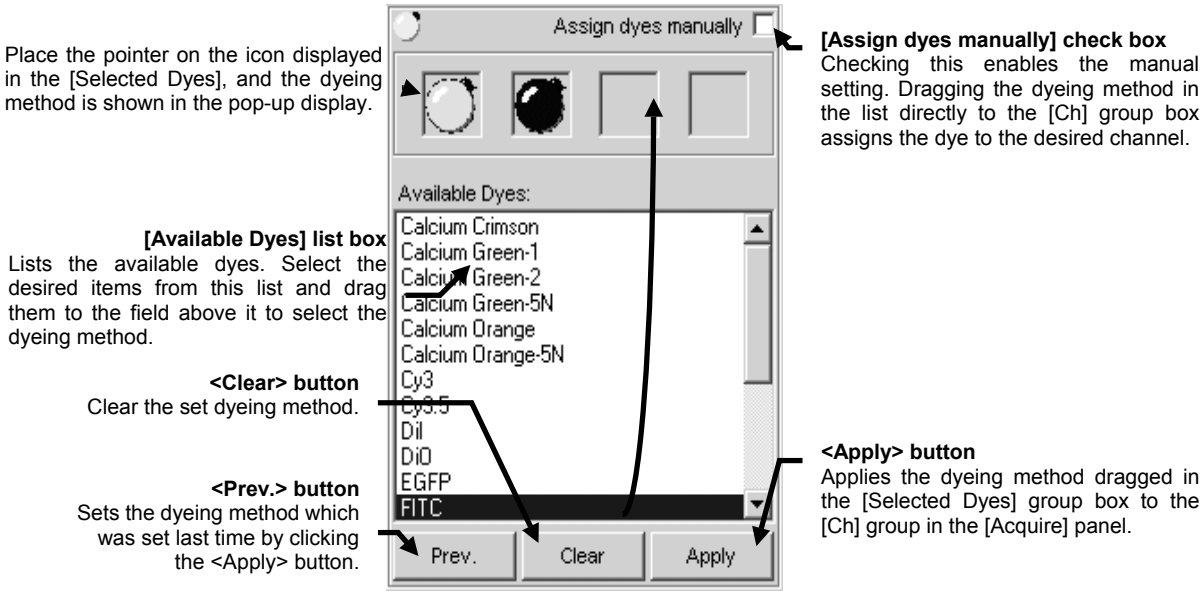


Fig. 1-6 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging [FITC] and [TRITC] in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.


Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.
[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.
[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.
<Clear> button
Clear the set dyeing method.
<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.
<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.
Assign dyes manually
Available Dyes:
Calcium Crimson
Calcium Green-1
Calcium Green-2
Calcium Green-5N
Calcium Orange
Calcium Orange-5N
Cy3
Cy3.5
DiI
DiO
EGFP
FITC
Prev.
Clear
Apply

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the switched filter. And the dyeing method is shown in the [Ch] group box.

The Confocal Aperture value is also set automatically according to the wavelength and the objective for every channel.

TIP

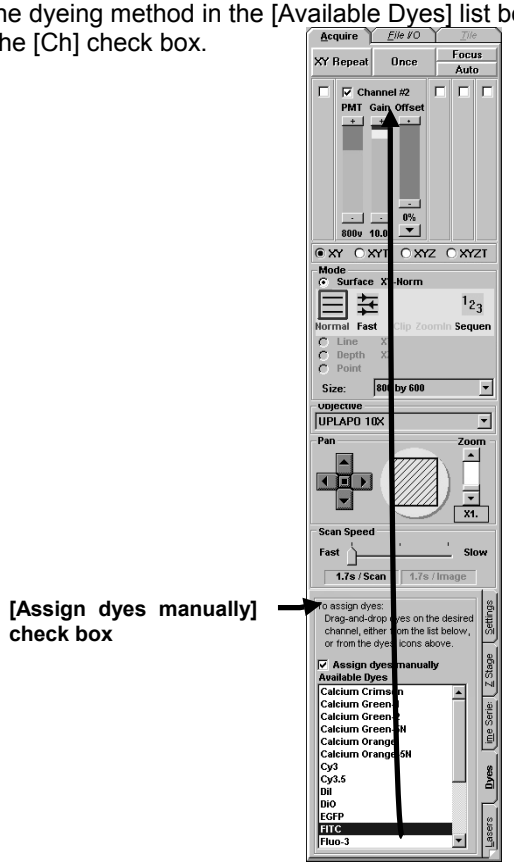
If you have changed the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.

For detailes, see section 1-3-2-4 “Configuring the Filters” for automatic Confocal Aperture setting with switching the objective.

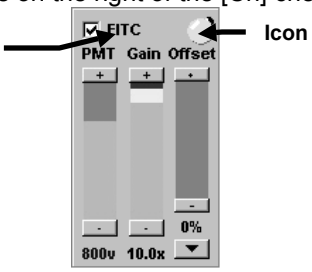
One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.



Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

1-2-5 Selecting the Filters

The excitation dichroic mirror, beam splitter and barrier filters are set automatically to the light path according to the dyeing method selected for the specimen.

To change filters, see section 1-3-2-4, “Configuring the Filters” in this volume and follow instructions in the [Optical System Configuration] window.

The following table shows the possible combinations of the barrier and excitation filters.

Laser Combination	Excitation Dichroic Mirror	Beam Splitter 1	Beam Splitter 2	Beam Splitter 3	Barrier Filter 1	Barrier Filter 2	Barrier Filter 3	Barrier Filter 4
Multiline Ar. HeNe(G), HeNe(R) 458/488/515, 543, 633 (3 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/543/633 (4) DM458/515 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -		(1) BA505IF (2) BA505-525 (3) BA480-495 (4) - (5) - (6) -	(1) BA560IF (2) BA560-620 (3) BA535-565 (4) - (5) - (6) -	(1) BA650IF (2) - (3) - (4) - (5) - (6) -	
Multiline Ar. HeNe(G), HeNe(R) 458/488/515, 543, 633 (3 channels) SPD system	(1) BS20/80 (2) DM405/488 (3) DM488/543/633 (4) DM458/515 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -		(1) BA505IF (2) BA505-525 (3) BA480-495 (4) - (5) - (6) -	(1) BA560IF (2) BA560-620 (3) BA535-565 (4) - (5) - (6) -	(1) BA650IF (2) BA560IF (3) - (4) - (5) - (6) -	
Multiline Ar, Kr, HeNe(R) 458/488/515, 568, 633 (3 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/568/633 (4) DM458/515 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -		(1) BA505IF (2) BA505-550 (3) BA480-495 (4) - (5) - (6) -	(1) BA585IF (2) BA585-615 (3) BA535-565 (4) - (5) - (6) -	(1) BA650IF (2) - (3) - (4) - (5) - (6) -	
Multiline Ar, Kr, HeNe(R) 458/488/515, 568, 633 (3 channels) SPD system	(1) BS20/80 (2) DM405/488 (3) DM488/568/633 (4) DM458/515 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -		(1) BA505IF (2) BA505-550 (3) BA480-495 (4) - (5) - (6) -	(1) BA585IF (2) BA585-615 (3) BA535-565 (4) - (5) - (6) -	(1) BA650IF (2) BA560IF (3) - (4) - (5) - (6) -	

Laser Combination	Excitation Dichroic Mirror	Beam Splitter 1	Beam Splitter 2	Beam Splitter 3	Barrier Filter 1	Barrier Filter 2	Barrier Filter 3	Barrier Filter 4
LD440, Multiline Ar, HeNe(G), HeNe(R) 440, 458/488/515, 543, 633 (3 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/543/633 (4) DM405-440/515 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -		(1) BA465-495 (2) BA505-525 (3) BA480-495 (4) - (5) - (6) -	(1) BA505IF (2) BA560-620 (3) BA535-565 (4) - (5) - (6) -	(1) BA650IF (2) BA560IF (3) - (4) - (5) - (6) -	
LD440, Multiline Ar, Kr, HeNe(R) 440, 458/488/515, 568,633 (3 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/568/633 (4) DM405-440/515 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -		(1) BA465-495 (2) BA505-550 (3) BA480-495 (4) - (5) - (6) -	(1) BA505IF (2) BA585-615 (3) BA535-565 (4) - (5) - (6) -	(1) BA650IF (2) BA585IF (3) - (4) - (5) - (6) -	
LD405, Multiline Ar, HeNe(G), HeNe(R) 405, 458/488/515, 543, 633 (3 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/543/633 (4) DM405-440/515 (5) DM405/488/543 (6) DM458/515	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) SDM490 (6) -	(1) Mirror (2) Glass (3) SDM640 (4) SDM560 (5) - (6) -		(1) BA465-495 (2) BA505-525 (3) BA430-470 (4) BA480-495 (5) - (6) -	(1) BA505IF (2) BA560-620 (3) BA535-565 (4) BA505-525 (5) - (6) -	(1) BA650IF (2) BA560IF (3) - (4) - (5) - (6) -	
LD405, Multiline Ar, Kr, HeNe(R) 405, 458/488/515, 568, 633 (3 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/568/633 (4) DM405-440/515 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) SDM490 (6) -	(1) Mirror (2) Glass (3) SDM640 (4) SDM560 (5) - (6) -		(1) BA465-495 (2) BA505-550 (3) BA430-470 (4) BA480-495 (5) - (6) -	(1) BA505IF (2) BA585-615 (3) BA535-565 (4) BA505-550 (5) - (6) -	(1) BA650IF (2) BA585IF (3) - (4) - (5) - (6) -	
LD405, Multiline Ar, HeNe(G), HeNe(R) 405, 458/488/515, 543, 633 (4 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/543/633 (4) DM405-440/515 (5) DM405/488/543 (6) DM458/515	(1) Mirror (2) Glass (3) SDM490 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) - (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -	(1) BA465-495 (2) BA430-470 (3) BA480-495 (4) - (5) - (6) -	(1) BA505IF (2) BA505-525 (3) BA535-565 (4) - (5) - (6) -	(1) BA560IF (2) BA560-620 (3) - (4) - (5) - (6) -	(1) BA650IF (2) - (3) - (4) - (5) - (6) -
LD405, Multiline Ar, Kr, HeNe(R) 405, 458/488/515, 568, 633 (4 channels)	(1) BS20/80 (2) DM405/488 (3) DM488/568/633 (4) DM405-440/515 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM490 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) - (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -	(1) BA465-495 (2) BA430-470 (3) BA480-495 (4) - (5) - (6) -	(1) BA505IF (2) BA505-550 (3) BA535-565 (4) - (5) - (6) -	(1) BA585IF (2) BA585-615 (3) - (4) - (5) - (6) -	(1) BA650IF (2) - (3) - (4) - (5) - (6) -

Laser Combination	Excitation Dichroic Mirror	Beam Splitter 1	Beam Splitter 2	Beam Splitter 3	Barrier Filter 1	Barrier Filter 2	Barrier Filter 3	Barrier Filter 4
UV, Multiline Ar, HeNe(G), HeNe(R) 351, 458/488/515, 543, 633 (3 channels)	(1) BS20/80 (2) DM351/488 (3) DM488/543/633 (4) DM351/543 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) SDM490 (6) -	(1) Mirror (2) Glass (3) SDM640 (4) SDM560 (5) - (6) -		(1) BA380-470 (2) BA505-525 (3) BA480-495 (4) - (5) - (6) -	(1) BA505IF (2) BA560-620 (3) BA535-565 (4) BA505-525 (5) - (6) -	(1) BA650IF (2) BA560IF (3) - (4) - (5) - (6) -	
UV, Multiline Ar, Kr, HeNe(R) 351, 458/488/515, 568, 633 (3 channels)	(1) BS20/80 (2) DM351/488 (3) DM488/568/633 (4) DM351/568 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM560 (4) SDM510 (5) SDM490 (6) -	(1) Mirror (2) Glass (3) SDM640 (4) SDM560 (5) - (6) -		(1) BA380-470 (2) BA505-550 (3) BA480-495 (4) - (5) - (6) -	(1) BA505IF (2) BA585-615 (3) BA535-565 (4) BA505-550 (5) - (6) -	(1) BA650IF (2) BA585IF (3) - (4) - (5) - (6) -	
UV, Multiline Ar, HeNe(G), HeNe(R) 351, 458/488/515, 543, 633 (4 channels)	(1) BS20/80 (2) DM351/488 (3) DM488/543/633 (4) DM351/543 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM490 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) - (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -	(1) BA380-470 (2) BA480-495 (3) - (4) - (5) - (6) -	(1) BA505IF (2) BA505-525 (3) BA535-565 (4) - (5) - (6) -	(1) BA560IF (2) BA560-620 (3) - (4) - (5) - (6) -	(1) BA650IF (2) - (3) - (4) - (5) - (6) -
UV, Multiline Ar, Kr, HeNe(R) 351, 458/488/515, 568, 633 (4 channels)	(1) BS20/80 (2) DM351/488 (3) DM488/568/633 (4) DM351/568 (5) DM458/515 (6) -	(1) Mirror (2) Glass (3) SDM490 (4) SDM510 (5) - (6) -	(1) Mirror (2) Glass (3) SDM560 (4) - (5) - (6) -	(1) Mirror (2) Glass (3) SDM640 (4) - (5) - (6) -	(1) BA380-470 (2) BA480-495 (3) - (4) - (5) - (6) -	(1) BA505IF (2) BA505-550 (3) BA535-565 (4) - (5) - (6) -	(1) BA585IF (2) BA585-615 (3) - (4) - (5) - (6) -	(1) BA650IF (2) - (3) - (4) - (5) - (6) -

- :Invaild

(1), (2), and (3) of the spectral filters are equipped as the factory configuration.

A single type of excitation filter can be equipped. Replace it if necessary.

Up to two types of barrier filter can be equipped per channel.

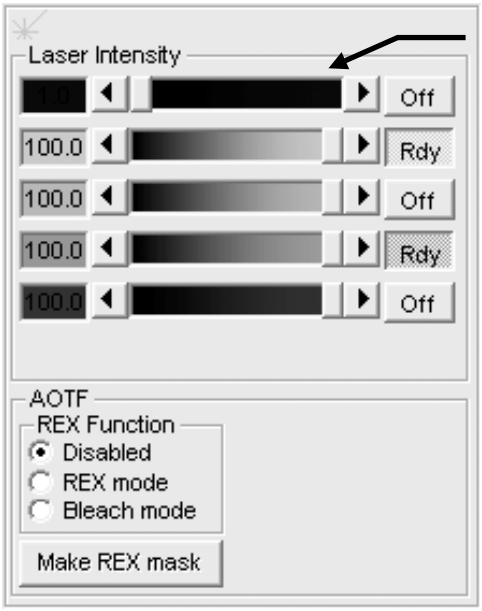
If the equipment of another filter set for laser configuration is required, please contact your local Olympus representative.

1-2-6 Setting the ND Filters

When you use the laser combiner, you can set the laser intensity by setting ND filter on the laser combiner.

Display the [Acquire] panel.

Set each laser intensity by sliding the scale bar in the [Laser Intensity] group box of the [Lasers] sub-panel, in accordance with specimen's brightness, fluorescence crosstalk and photo-bleaching.



[Laser Intensity] group box
Set the laser intensity value by the scale bar..
The number of the displayed laser intensity sliders varies depending on that of channels setting for the acquisition.

Fig. 1-7 **[Lasers] Sub-panel**

While using the HeNe green laser, try out the laser power 50% by setting the [Intensity] scale bar in the [Laser Intensity] group box .

For other lasers, try the laser power 5% .

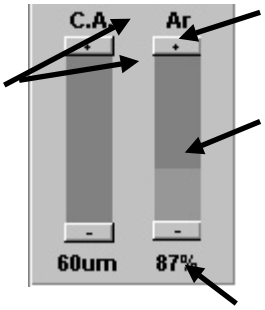
One Point!

After the dyeing method has been set with the [Dyes] sub-panel, the ND filters can be set using the [Ch] group box in the upper part of the [Acquire] panel.

1. In the upper part of the [Acquire] sub-panel, open the [Ch] group box for the ND to be changed.
2. Click the <More> button.

   The field as shown below is displayed below the [Ch] group box.

Display of the optimum laser and ND value which are set automatically according to the selected dyeing method.



Clicking this button allows fine adjustment of the ND value.

Clicking this field allows the ND value to be changed on a large scale.
The ND values which are usually used are displayed in green.

Display of the ND value set by clicking the <+> and <-> buttons or the field. The set ND value can also be changed by entering its value directly from the keyboard.

3. Vary the ND value using the Laser LED slider.

   Each click of the laser ND <+> or <-> varies the laser ND value by 1%.

   Each click of the laser ND slider varies the laser ND value by 5%.

1-2-7 Setting the Observation Condition

1-2-7-1 Setting the Objective Magnification

From the drop-down list on the center of the [Acquire] panel, select the objective being used with the microscope.

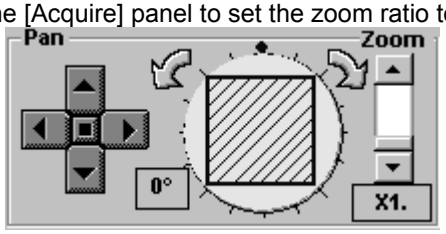
TIP

If you change the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.

For detailes, see section 1-3-2-4 “Configuring the Filters” for automatic Confocal Aperture setting with switching the objective.

1-2-7-2 Setting the Zoom Ratio to 1X

Use the [Zoom] scale in the [Acquire] panel to set the zoom ratio to “X1”.



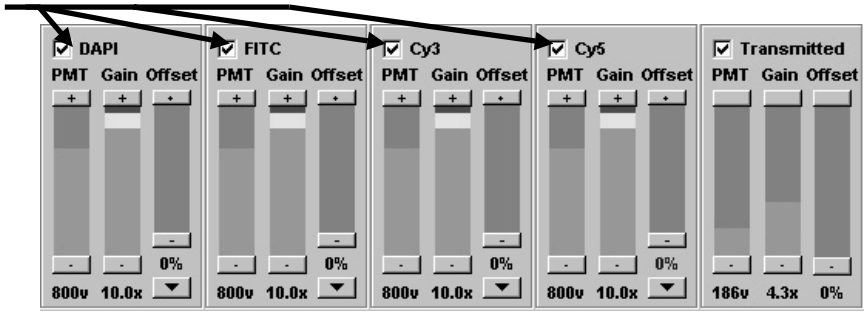
Using the UV-Ar laser, set the zoom ratio to “X2”.

1-2-7-3 Setting the Channels

1. In the [Ch] group box, make sure that the check boxes showing the applicable dyeing methods are check-marked to indicate that the channels are ready for image acquisition.

   If the check box of a channel is not check-marked, check it to make the channel ready.

Channel check boxes, with which dyeing methods are displayed



TIP

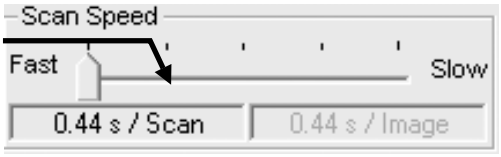
To display the information on all channels simultaneously, right-click the boundary between channel display boxes.

Click the boundary again to return to the original display.

1-2-7-4 Setting the Highest Scan Speed

1. Set the scan speed to the fastest speed by using the scale in the [Scan Speed] group box in the [Acquire] panel

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



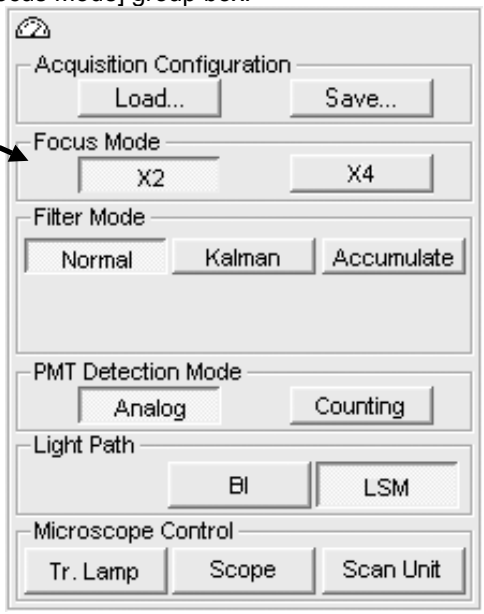
TIP

The focus mode makes it possible to increase the line skipped scan speed.

From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel.

Select either option button in the [Focus Mode] group box.

[Focus Mode] group box
[X2] option button
Acquires image at twice the highest speed.
[X4] option button
Acquires image at 4 times the highest speed.
Increasing the number of divided images in the image window, line skipped scan at 4 times (Focus) cannot be done.



The focus mode is enabled when acquiring images using the <Focus> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

1-2-7-5 Setting the XY Observation Mode

1. In the [Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Mode] group box in the [Acquire] panel, select [800 by 600] from the [Scan Size] drop-down list.
3. In the [Acquire] panel, select the XY observation mode option button.

1-2-7-6 Repeated Scanning Operation

<XY Repeat> button

<Focus> button

1. Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel.

TIP

Use the <FOCUS> button to acquire image at an even higher speed. If the specimen is already being scanned, stop scanning with the <STOP SCAN> button before selecting the <XY Repeat> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

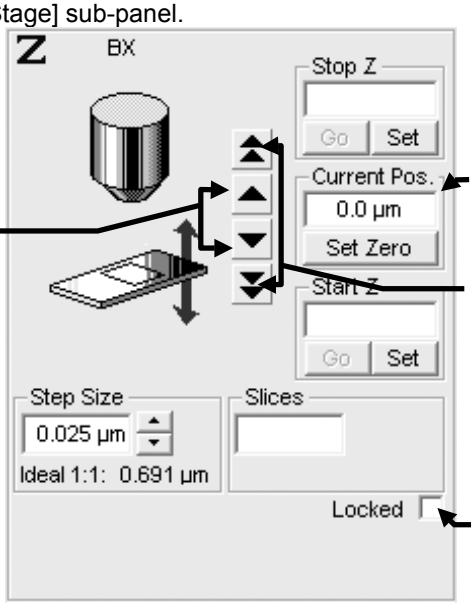
NOTE

Do not move FLOUVIEW FV1000 Menu while acquiring an image.

1-2-7-7 Setting the Cross-section to be Observed

While acquiring image, move the Z stage to select the cross-section to be observed.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



<Z stage fine adjustment> buttons
Displace the Z stage by 0.1 µm per step.

[Current Pos] text box
Shows the current position of the stage. The value can also be entered directly from the keyboard.

<Z stage coarse adjustment> buttons
Displace the Z stage by 1.0 µm per step.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 1-8 **[Z Stage] Sub-panel**

2. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

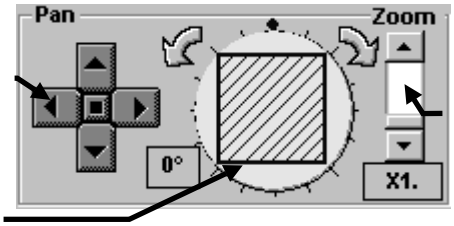
3. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

1-2-7-8 Setting the Area to be Observed

When the observation targets are concentrated in a narrow area or when observation of a specific area detail is required, the image of a limited area can be selected.

The 4 buttons represent directions, and clicking a button moves the acquired image area in the direction indicated by the button.
Clicking the square button on the center returns the acquired image area to the center.

Click a point inside the circle to change the position of the acquired image area directly.



Clicking a point in the scale area to change the value on a large scale.
Clicking the top or bottom arrow button allows fine adjustment of the value.
Dragging the square knob allows the value to be changed directly.

Fig. 1-9 [Pan]/[Zoom] Group Box

1-2-7-9 Setting a Lower Scan Speed

The scan speed can be decreased using the scale in the [Scan Speed] group box on the [Acquire] panel.

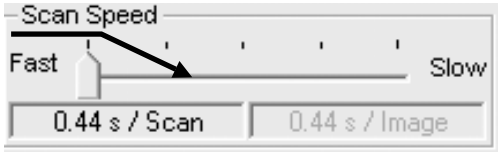
TIP

In general, setting a lower scan speed allows the acquired image quality to be improved.
However, a low scan speed also lengthens the time required for image acquisition.

NOTE

When the scan speed is decreased during fluorescence observation, the saturation of fluorescence may darken the image of certain types of specimens. In this case, increase the scan speed and increase the PMT Voltage or use accumulation in scanning.

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



1-2-7-10 Stopping Repeated Scanning

After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily.

1-2-8 Acquiring Image

<Once> button

Select the <Once> button. The acquired image will be displayed in the [Live] panel.

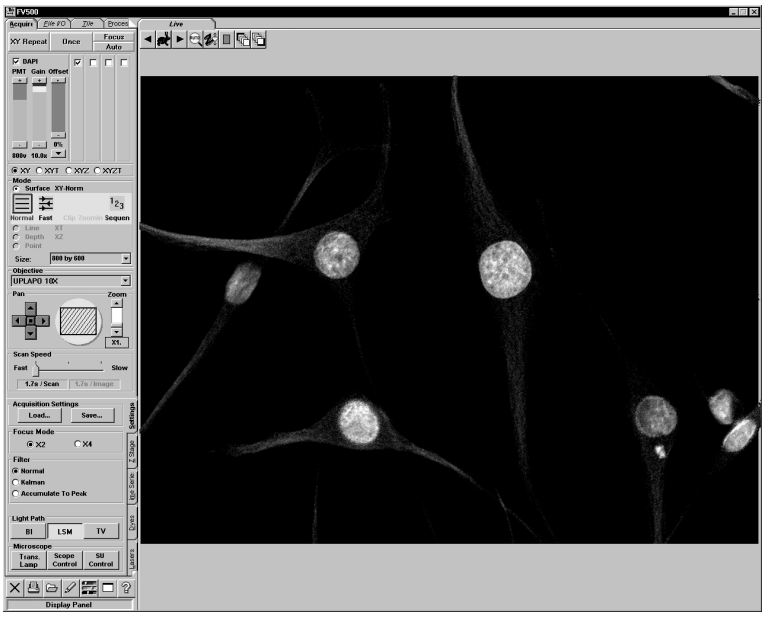
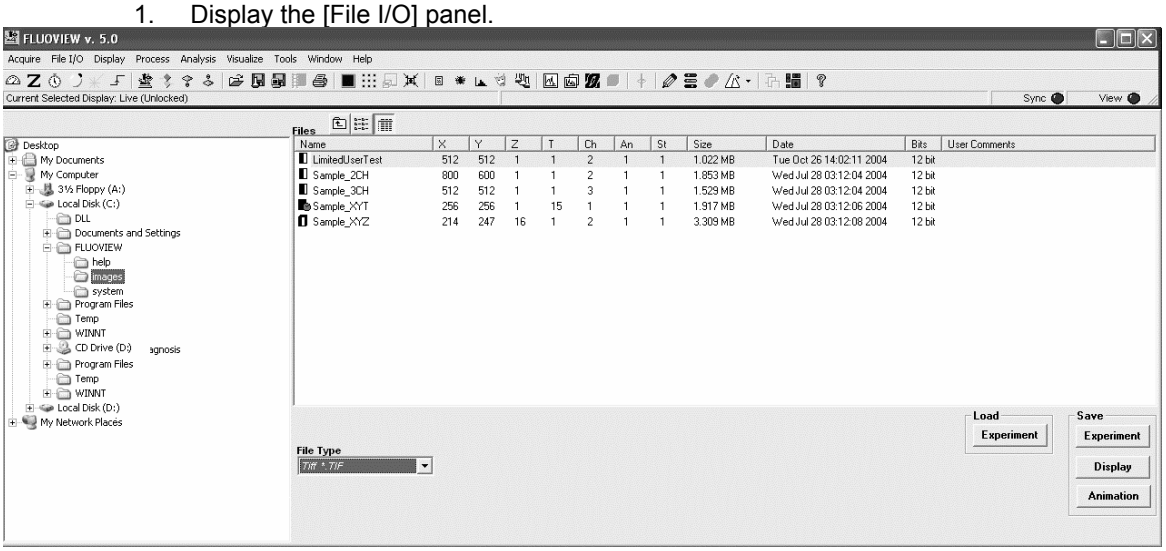
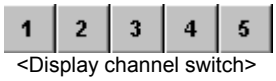


Fig. 1-10 Image Acquired in the [Live] Panel

1-2-9 Saving Image

1. Display the [File I/O] panel.


FLUOVIEW v. 5.0
Acquire File I/O Display Process Analysis Visualize Tools Window Help
Current Selected Display: Live (Unlocked)
Sync
View
Files
Desktop
My Documents
My Computer
3½ Floppy (A:)
Local Disk (C:)
DLL
Documents and Settings
FLUOVIEW
help
Images
system
Program Files
Temp
WINNT
CD Drive (D:)
Program Files
Temp
WINNT
Local Disk (D:)
My Network Places
Name X Y Z T Ch An St Size Date Bits User Comments
LimitedUserTest 512 512 1 1 2 1 1 1.022 MB Tue Oct 26 14:02:11 2004 12 bit
Sample_2CH 800 600 1 1 2 1 1 1.853 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_3CH 512 512 1 1 3 1 1 1.529 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_XYT 256 256 1 15 1 1 1 1.917 MB Wed Jul 28 03:12:06 2004 12 bit
Sample_XYZ 214 247 16 1 2 1 1 3.309 MB Wed Jul 28 03:12:08 2004 12 bit
Load
Experiment
Save
Experiment
Display
Animation
File Type
TIF *.TIF



<Display channel switch> buttons

2. When saving images acquired with more than one channel, it is possible to select whether images from more than one image are saved simultaneously or only one of the images is saved.

   Use the <Display channel switch> buttons to select the images to be saved. The selected images will be saved under the conditions set for each channel.

   Example)When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.

3. Click <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box will open.

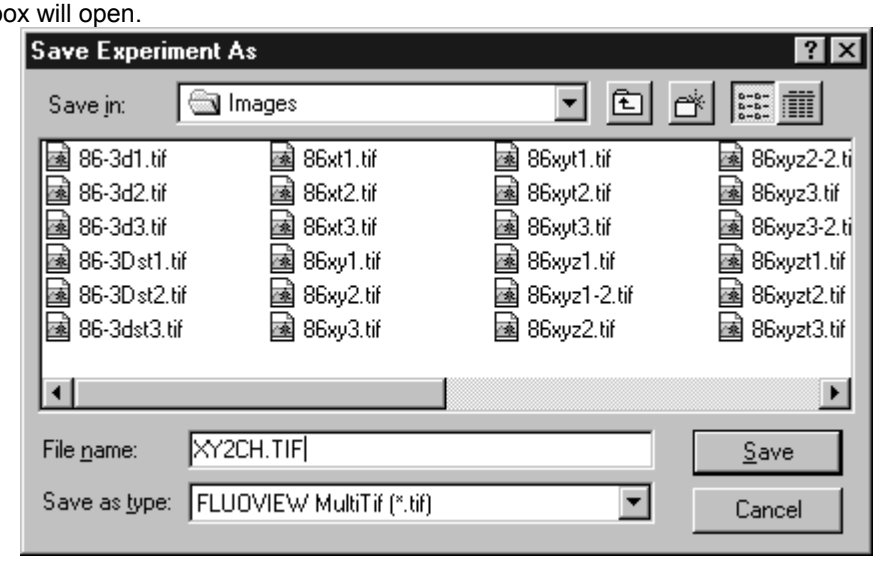


Fig. 1-11 [Save Experiment As] Dialog Box

4. Enter the file name in the [File name:] text box.

5. Select “FLUOVIEW MultiTif” from [Save as type:]

6. Click the <Save> button.

1-2-10 Exiting from the Software, Turning Power Off

Exit from the software, then set the power switch of each unit to off.

For details, see section 1-3 “Exiting from the Software” and section 1-4 “Turning the Power Off” in Volume II PREPARATION For OPERATION of [HARDWARE GUIDE] of the FV1000 User’s Manual.

1-3 Online Help

The FLUOVIEW application comes with two kinds of online help facility:

- function help for referencing the function and operation procedure description while controlling the application, and
- microscope help providing information on the system setup.

1-3-1 Function Help

This section describes a simple method for displaying and consulting the online help on the functions and operation procedures.

The figure below shows the initial display (table of contents) of the FLUOVIEW Online Help window. To display this window, click the <Help> button in the toolbar at the bottom left of the screen.

<Help> button

Some words in the displayed information are shown in enhanced display (underscored or colored green). Clicking one of these words allows you to "jump" to the meaning of the word or to more information about its meaning.

Finger pointer

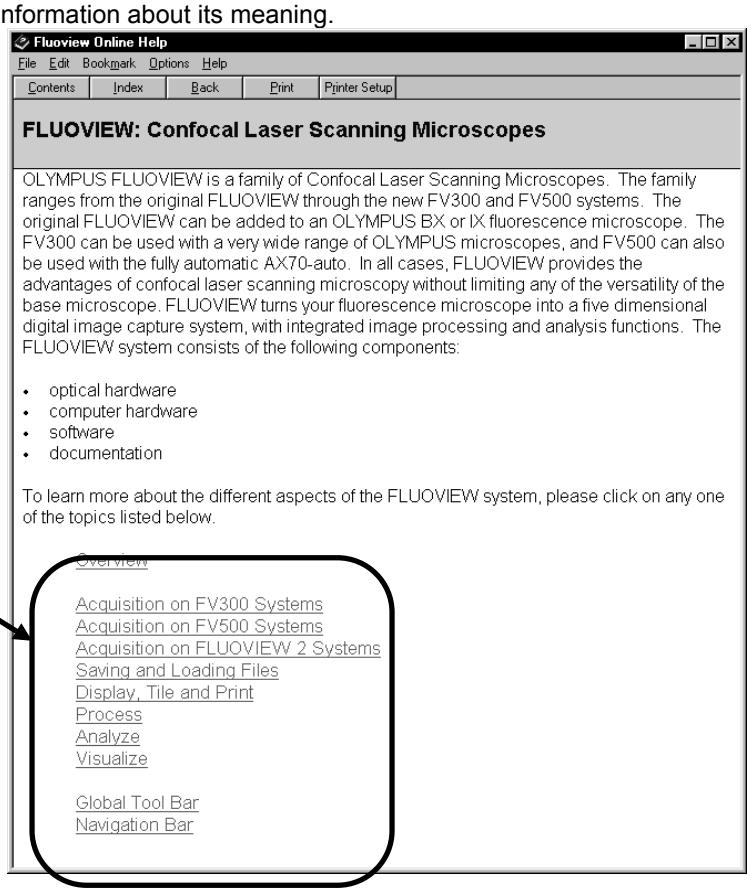

Fluoview Online Help
File Edit Bookmark Options Help
Contents Index Back Print Printer Setup
FLUOVIEW: Confocal Laser Scanning Microscopes
OLYMPUS FLUOVIEW is a family of Confocal Laser Scanning Microscopes. The family ranges from the original FLUOVIEW through the new FV300 and FV500 systems. The original FLUOVIEW can be added to an OLYMPUS BX or IX fluorescence microscope. The FV300 can be used with a very wide range of OLYMPUS microscopes, and FV500 can also be used with the fully automatic AX70-auto. In all cases, FLUOVIEW provides the advantages of confocal laser scanning microscopy without limiting any of the versatility of the base microscope. FLUOVIEW turns your fluorescence microscope into a five dimensional digital image capture system, with integrated image processing and analysis functions. The FLUOVIEW system consists of the following components:
optical hardware
computer hardware
software
documentation
To learn more about the different aspects of the FLUOVIEW system, please click on any one of the topics listed below.
Overview
Acquisition on FV300 Systems
Acquisition on FV500 Systems
Acquisition on FLUOVIEW 2 Systems
Saving and Loading Files
Display, Tile and Print
Process
Analyze
Visualize
Global Tool Bar
Navigation Bar
Enhanced display

Fig. 1-13 Initial Window

TIP

When the mouse pointer is placed on a word in enhanced display, the mouse pointer turns into a “finger pointer”.

One Point!

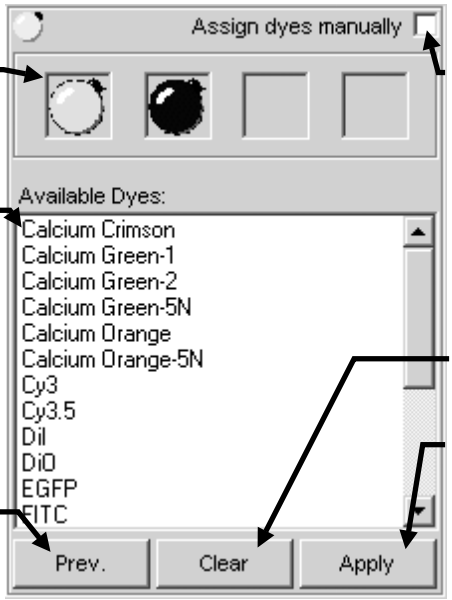
Click the <Contents> button to the initial display. Click the <Back> button to return to the previous information page.

1-3-2 Microscope Help

The microscope, scan unit and laser types can be set up from the FLUOVIEW software, by selecting the dyeing method and following the displayed guidance information.

- Selecting the Dyeing Method

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method directly to the [Ch] group box assigns the dyes to the desired channel.

[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Clear> button
Clear the set dyeing method.

<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.

Fig. 1-14 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

TIP

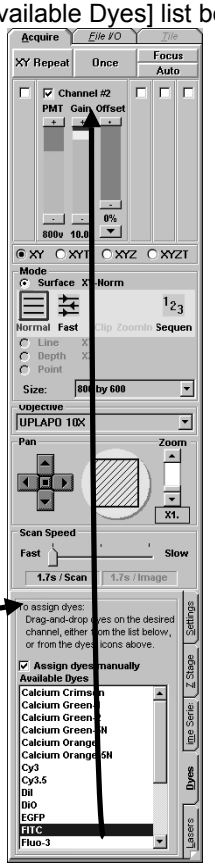
When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

The Confocal Aperture value is also set automatically according to the wavelength and the objective per channels.

One Point!

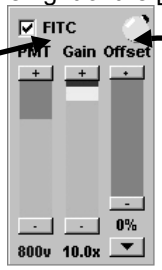
The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.



The dyeing method is set

Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

1-3-2-1 Configuring the Microscope

When the combination with BX61 or IX81 is in use, the microscope and scan unit can be configured on the FLUOVIEW software.

Use the following procedure to configure the microscope.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

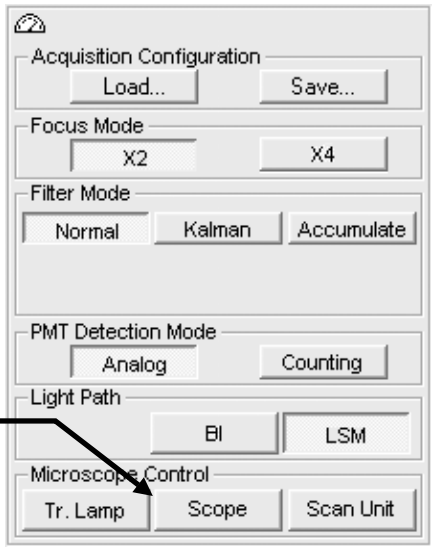
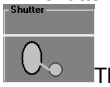


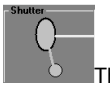
Fig. 1-15 [Settings] Sub-panel

2. Select the <Scope> button on the bottom of the [Settings] sub-panel. The [Microscope Control] window as shown below appears.

[Shutter] group box
Clicking inside the box switches the EPI shutter to be closed/opened,



The shutter is opened.



The shutter is closed.

[EPI lamp]
Indicates the EPI lamp.

[Filter Turret] group box
Clicking the filter for reflected light observation to be set switches the turret automatically.

[Light Path] group box
Selects the light path. <BI> is for direct observation, <LSM> is for LSM observation and <TV> is for TV observation.

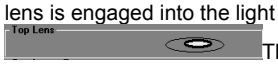
[Filter Turret] group box
Clicking the filter for visual observation to be set switches the turret automatically.

[Mirror Unit] group box
Clicking the cube automatically switches the turret.

[Nosepiece] group box
Click to change the objective.

[Top Lens] group box
Clicking inside the box switches the top lens to be engaged into the light path.

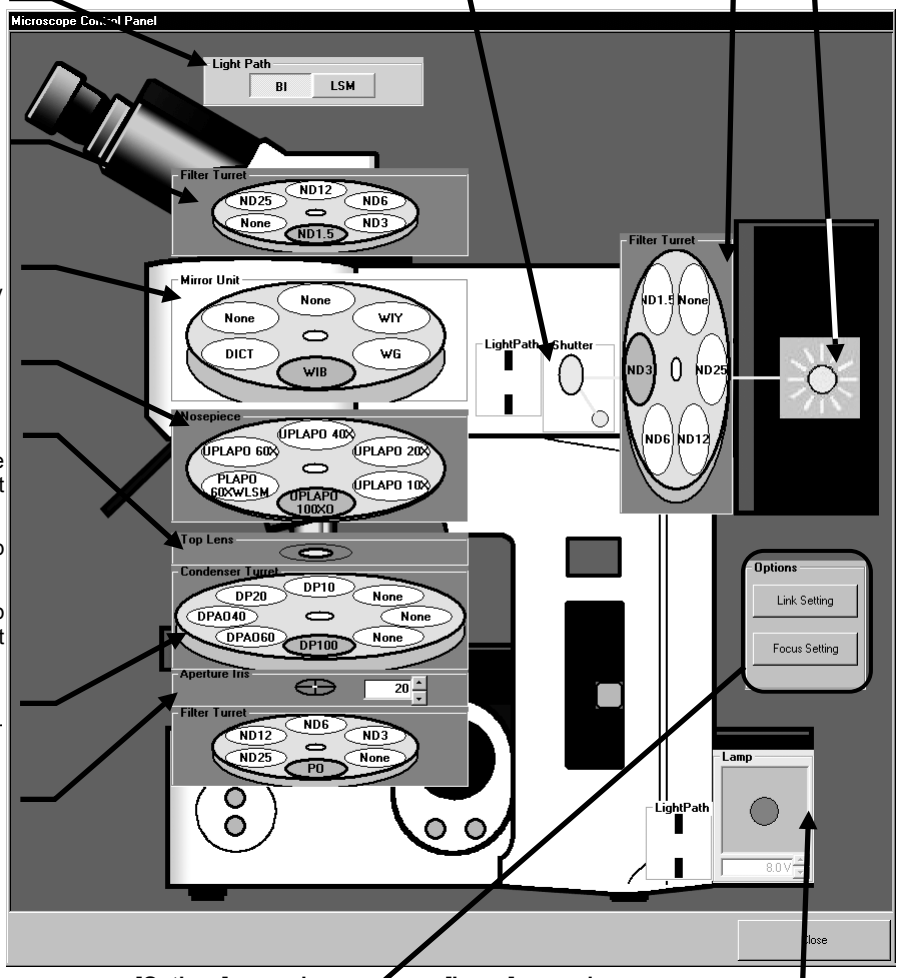
The top lens is engaged into the light oath.



The top lens is disengaged from the light path.

[Condenser Turret] group box
Clicking the universal condenser automatically switches the turret.

[Aperture Iris] group box
Changes the AS value.



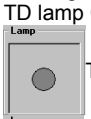
[Options] group box
Used for optional BX settings.

<Link Setting> button
Selects the function to change settings corresponding to the change of BX settings.

<Focus Setting> button
Enables parfocal corrections and adjustments of jogging sensitivity for the objectives.

(Combination with BX)

[Lamp] group box
Clicking inside the box switches the TD lamp ON/OFF.

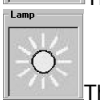


The TD lamp is set to OFF.

The TD lamp is set to ON.

[Lamp] group box
Clicking inside the box switches the TD lamp ON/OFF.

The TD lamp is set to OFF.



The TD lamp is set to ON.

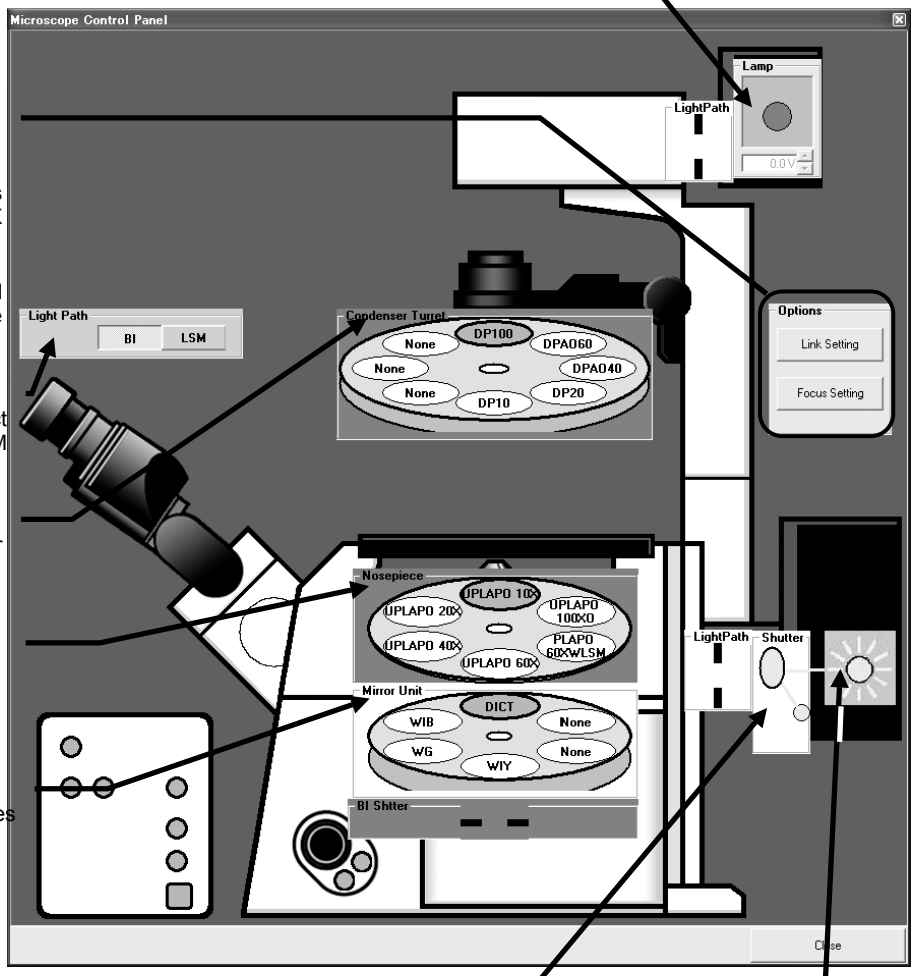
[Options] group box
Used for optional IX settings.
<Link Setting> button
Selects the function to change settings corresponding to the change of IX settings.
<Focus Setting> button
Enables parfocal corrections and adjustments of jogging sensitivity for the objectives.

[Light Path] group box
Selects the light path. <BI> is for direct observation, <LSM> is for LSM observation.

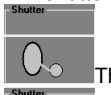
[Condenser Turret] group box
Clicking the universal condenser automatically switches the turret.

[Nosepiece] group box
Click to change the objective.

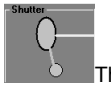
[Mirror Unit] group box
Clicking the cube automatically switches the turret.



[Shutter] group box
Clicking inside the box switches the EPI shutter to be closed/opened,



The shutter is opened.



The shutter is closed.

[EPI lamp]
Indicates the EPI lamp.

(Combination with IX)

- Clicking the <Link Setting> button displays the [Link Setting] dialog box as shown below.

Checking here links the objective in the [Nosepiece] group box with the condenser turret.

Checking here escapes the stage or revolving nosepiece when the objective is selected and changed in the [Nosepiece] group box

Checking here disengages the top lens from the light path when the objective of ×4 or lower magnification is selected in the [Nosepiece] group box. (BX only)

Checking here enables parfocality correction when the objective is selected and changed in the [NosePiece] group box.

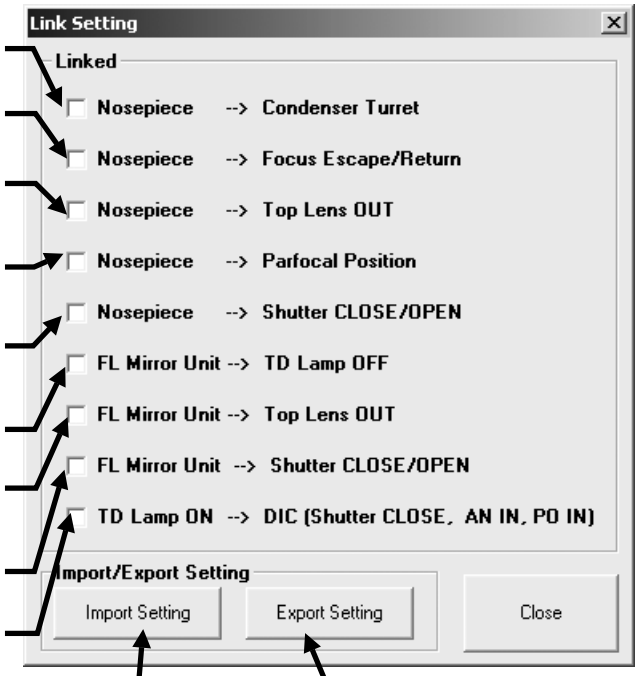
When objective is selected in [Nosepiece] group box while this box is checked, close shutter of incident lighting lamp first and then, change objective lens. After change, open the shutter.

Checking here sets the TD lamp to OFF when the fluorescent cube is selected in the [Mirror Unit].

Checking here disengages the top lens from the light path when the fluorescent cube is selected in the [Mirror Unit]. (BX only)

Checking here closes the shutter when the Mirror Units are switched in the [Mirror Unit].

Checking here closes the FL shutter and engages Analyzer and Polarizer into the light path when the TD lamp is set to ON in the [Lamp] group box.


Link Setting
Linked
Nosepiece --> Condenser Turret
Nosepiece --> Focus Escape/Return
Nosepiece --> Top Lens OUT
Nosepiece --> Parfocal Position
Nosepiece --> Shutter CLOSE/OPEN
FL Mirror Unit --> TD Lamp OFF
FL Mirror Unit --> Top Lens OUT
FL Mirror Unit --> Shutter CLOSE/OPEN
TD Lamp ON --> DIC (Shutter CLOSE, AN IN, PO IN)
Import/Export Setting
Import Setting
Export Setting
Close

<Import Setting> button
Loads the microscope settings already registered to reflect it.

<Export Setting>button
Saves the current

Click the desired check box to be checked.

Select the <Close> button to close the dialog box.

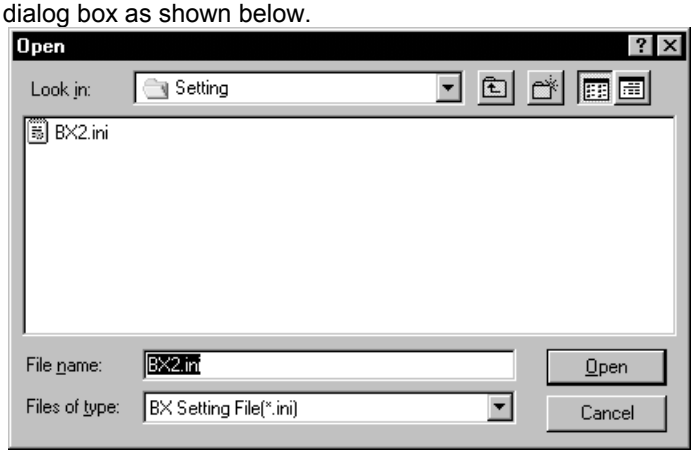
The microscope settings are automatically saved and read out when the software is started up next time.

Open the setting

Open the file to which the microscope settings were exported with the <Export Setting> button.

The Settings configured by another user or configured for another combination can be applied.

Selecting the <Import Setting> button of the [Link Setting] dialog box displays the [Open] dialog box as shown below.



When the setting file which you want to read is not displayed in the list box, use the [Look in:] drop-down list and select the drive or directory where the file is saved.

Select “BX Setting File (*.ini)” in the [Files of type:] drop-down list.

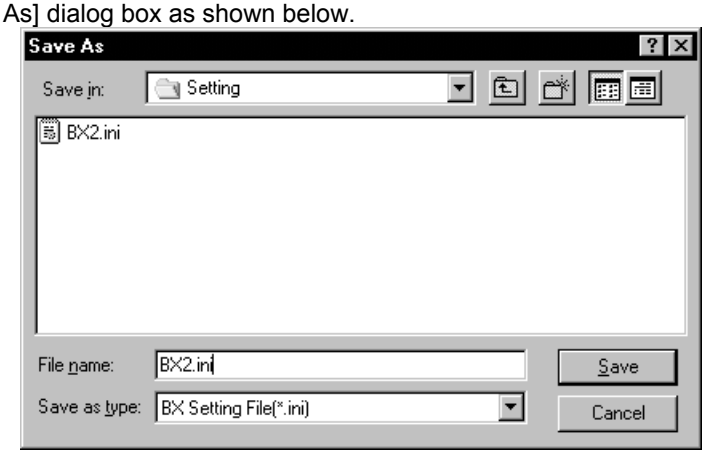
In the list box, select the setting file which you want to read.

Select the <Open> button to close the dialog box.

Save the setting

Settings in the [Link Setting] dialog box can be applied to other users or other combinations.

Selecting the <Export Setting> button of the [Link Setting] dialog box displays the [Save As] dialog box as shown below.



To change the save destination drive or directory, use the [Save in:] drop-down list.

Enter the setting file name into the [File name] text box.

Select the <Save> button to close the dialog box.

- The <Focus Setting> button enables parfocal corrections and adjustments of jogging sensitivity for the objectives.

  See section 1-3-2-2, “Parfocality Correction and Jog Sensitivity Adjustment”.

  Select the <Exit> button to close the dialog box.

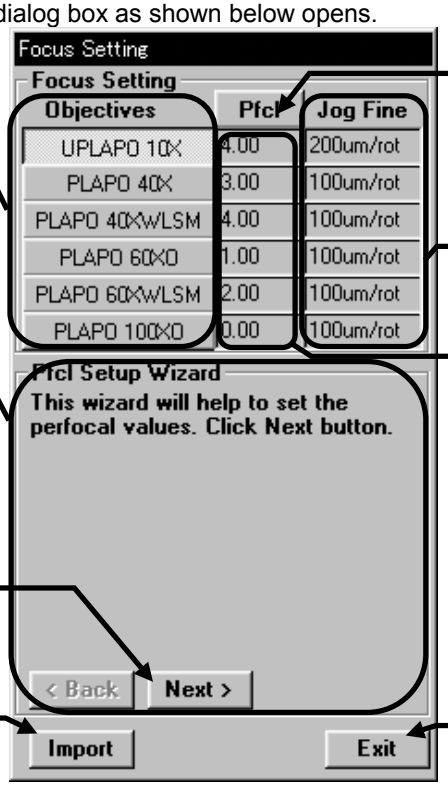
3. After completing the system setup, click the <Close> button to close the window.

1-3-2-2 Parfocality Correction and Jog Sensitivity Adjustment

When the system use the BX or IX microscope, the parfocality correction and jog sensitivity can be set per objective.

1. Open the [Microscope Control Panel] window.
   For the method of displaying this window, see steps 1 and 2 in section 1-3-2-1, “Configuring the Microscope ” in this section.

2. Click the <Focus Setting> button in the [Microscope Control Panel] window.
   The [Focus Setting] dialog box as shown below opens.



<Pfcl> button
Depress to activate the parfocality correction. To deactivate, depress this button once again.

[Objectives] group box
Click the button for the objective subjected to parfocality correction so that the button looks depressed.

[Jog Fine] group box
Select the jog sensitivity of each objective from the drop-down list.

Display of the parfocality correction value of each objective.

[Pfcl Setup Wizard] group box
The Wizard for parfocality correction.

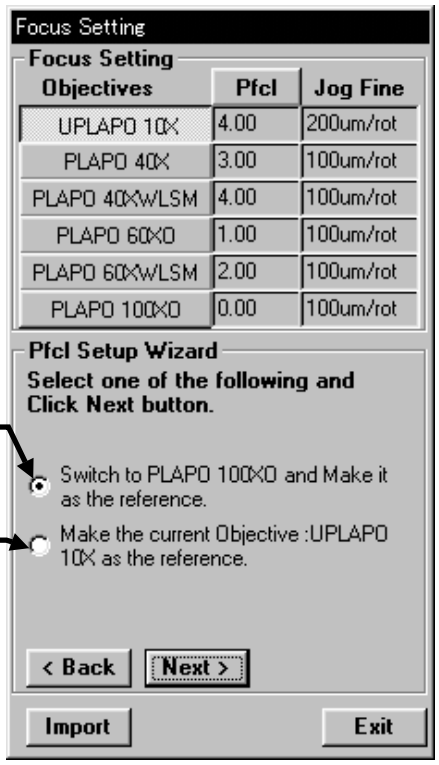
<Next> button
Click to execute the Parfocality Setup (correction) Wizard.

<Import> button
Click to import the previously saved parfocality correction and jog sensitivity values for each objective.

<Exit> button
Click to close the [Focus Setting] dialog box.

3. When the [Focus Setting] dialog box is displayed, the objective mounted on the microscope is selected.
   To use the setup wizard to begin the parfocality correction from the value of the objective with maximum magnification in descending order, click the <Next> button in the [Pfcl Setup Wizard] group box.

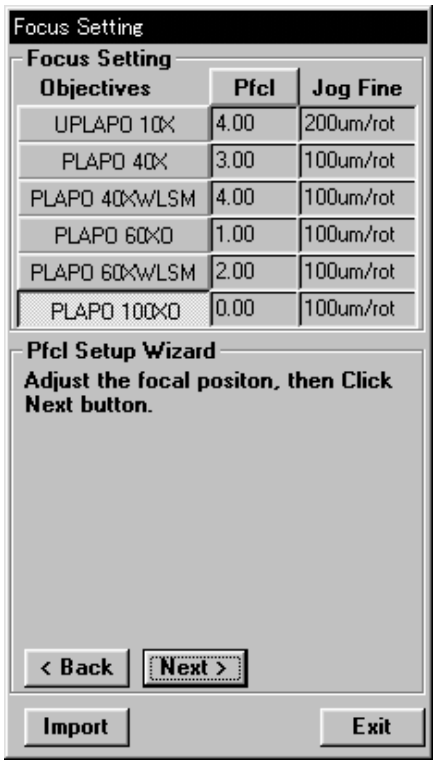
4. The following option button menu is displayed in the [Pfcl Setup Wizard] group box. If you want to begin the parfocality correction value setting with the objective with the maximum magnification, click the [Switch to...] option button then click the <Next> button.



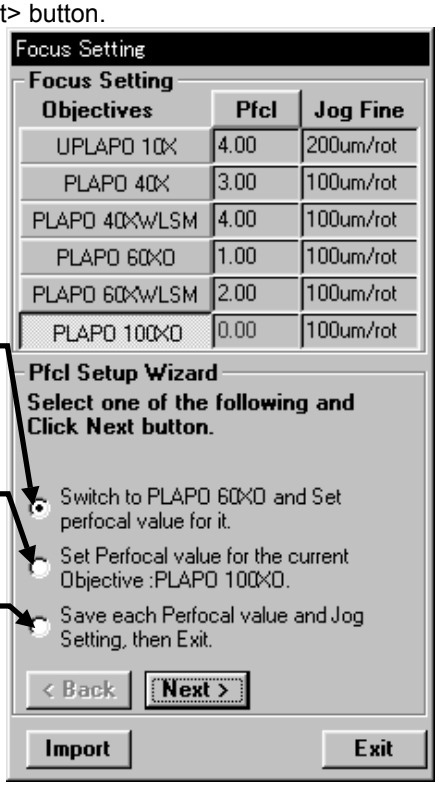
Click to set the objective with the maximum magnification in the list as the reference for correction of other objectives.

Click to set the objective being selected in the [Objectives] group box as the reference for correction.

5. The objective set as the specified in the [Objectives] group box is selected, and the [Pfcl Setup Wizard] group box displays the message shown below. Bring the specimen in focus by observing it visually or on the scanned image, and then click the <Next> button.



6. The [Pfcl Setup Wizard:] group box displays the option button menu shown below. If you want to set the parfocality correction values of objectives with other power values, simply click the <Next> button.

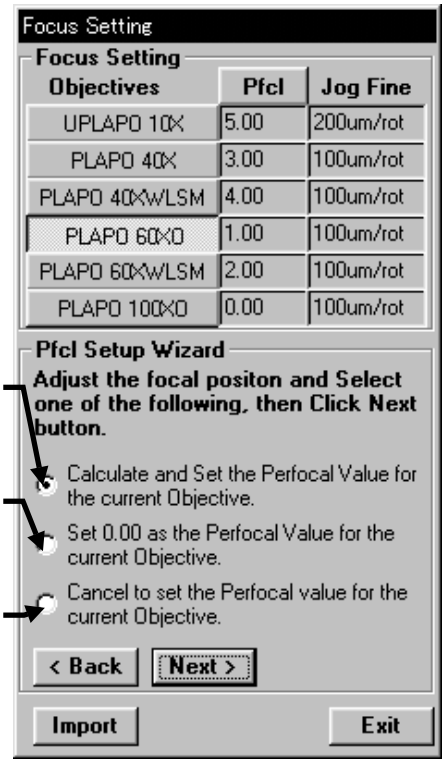


Click to proceed to the setting of the objective with next higher power to the current objective.

Click to select the desired objective from the [Objectives] group box and set the parfocality correction value for it.

Click to save the currently set parfocality correction and jog sensitivity values, then close the dialog box.
(This option button is displayed when the setting is changed.)
(Click the <Next> button to save the setting and close the dialog box.)

7. The [Pfcl Setup Wizard] group box displays the option button menu shown below. Adjust the focal position and click the <Next> button.



Click to set the current focal position as the parfocality correction value for the current objective and proceed to the setting of the next objective.

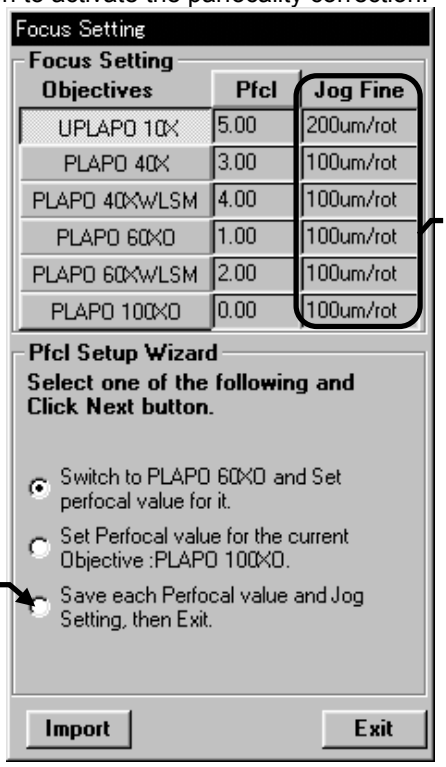
Click to set the parfocality correction value of the current objective to “0.00” and proceed to the setting of the next objective.

Click to proceed to the parfocality correction setting of the next objective without changing the setting for the current objective and jog sensitivity values.

8. Set the parfocality correction values of objectives by repeating steps 6 and 7 for each.

   The [Pfcl Setup Wizard] group box displays the option button menu shown below.

   Click the <Pfcl> button to activate the parfocality correction.



[Jog Fine] group box
Select the jog sensitivity of each objective from the drop-down list.
The * mark represents the value recommended.

Click to save the currently set parfocality correction and jog sensitivity values, then close the dialog box.
(This option button is displayed when the setting is changed.)
(Click the <Next> button to save the setting and close the dialog box.)

9. Set the jog sensitivity values of the objectives.

   Select the jog sensitivity of each objective from the [Jog Fine] drop-down list.

   The drop-down list appears when clicking each value in the [Jog Fine].

10. After completing the setting, click the [Save each Perfocal value and Jog Setting, then Exit] option button and then click the <Next> button.

    The setting will be registered and the dialog box will close.

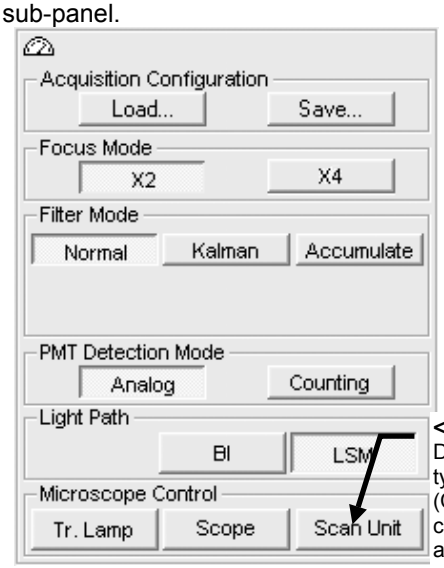
    If you do not want to register the setting, simply click the <Exit> button to close the dialog box.

1-3-2-3 Configuring the Filters (When using a filter system)

The barrier filters, excitation filter and beam splitter are set automatically to the light path according to the dyeing method selected for the specimen.

Use the following procedure to change these filters.

1. Select the [Settings] sub-panel.



<Scan Unit> button
Display the filter and laser types to be set.
(Combination with the laser combiner sets up the laser automatically.)

Fig. 1-16 [Settings] Sub-panel

2. Click the <Scan Unit> button at the bottom of the panel. The window as shown below will appear.

[Laser Unit] group box
Shows the type of laser to be used. (With the laser combiner operation, the laser type is set and displayed automatically.) When the <On> button is depressed, the laser is oscillating the beam. When the <Stby> button is depressed, the laser is not oscillating. When the [Auto standby] check box is checked, the [After] text box appears below it. When not using laser for a long time, in order to suppress useless electric-power consumption, we recommend you making <Stby> mode. The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser is suspended as standby mode (using Ar or Kr-laser) or the laser oscillation stops (using UV-Ar laser) When the time shown in this box has elapsed after the end of laser scanning.

[Excitation DM] and [Beam splitter] drop-down lists
Change the excitation filter and beam splitter of each channel.

Clicking this area causes the [Microscope Configuration] window to appear.

[Fit C.A. for change of Objective Lens] Check box
In case of check this box, Confocal Aperture diameter is set with switching the objective.

[TD Unit] group box
Shows the transmitted light detection.

[Barrier Filter] drop-down list
Changes the barrier filter of each channel.

[Dyes]
Shows the dyeing method set for each channel.

[XY Resolution]
Shows lateral resolution for each channel,

[Z Resolution]
Shows the Z resolution set for each channel.

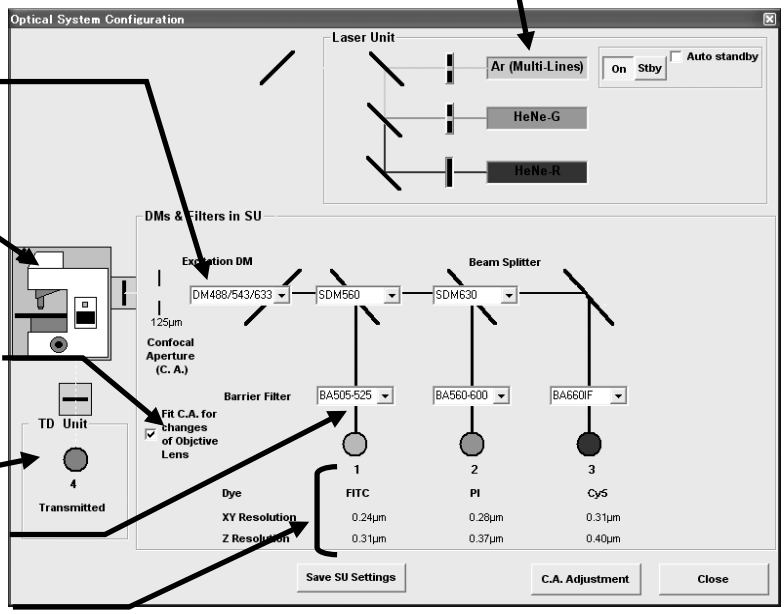


Fig. 1-17 [Optical System Configuration] Window

TIP

Virtual channels can be used. For the virtual channels, see section 2-2-8-1, "Virtual Channel".

3. To change the displayed filter types, use the [Excitation DM], [Beam splitter] and/or [Barrier Filter] drop-down lists.

4. After completing the fitler setup, click the <Close> button to close the window.

One Point!

The formulas of resolution of X and Y are as follows;

FWHM : Resolution of X and Y

L : The length of a diagonal line of Square Pinhole

NA = nSINθ :NA of Objective lens

MG : The objective Magnification

N : The refractive index of a medium

Air : 1.0

Water : 1.3

Oil : 1.5

$X = L/(1.22*3.8*\lambda*MG/NA)$

The case where the variable x is as follows; 0 <= x < 0.5

FWHM = (0.5λ*XYPSF)/NA

$XYPSF = -5.1459x^6 + 1.0147*10x^5 - 7.1668x^4 + 2.2804x^3 - 1.7696*10^{-1}x^2 + 1.9256*10^{-02}x + 7.1915*10^{-1}$

The case where the variable x is as follows; 0.5 <= x < 1.0

FWHM = (0.5λ*XYPSF)/NA

$XYPSF = -3.05539x^6 + 1.32031*10x^5 - 2.25074*10x^4 + 1.89617*10x^3 - 7.89685x^2 + 1.56587x + 6.20850*10^{-1}$

The case where the variable x is as follows; 1.0 <= x < 1.7

FWHM = (0.5λ*XYPSF)/NA

$XYPSF = -2.61786x^6 + 2.14478*10x^5 - 7.18428*10x^4 + 1.25767*10^2x^3 - 1.21445*10^2x^2 + 6.17335*10x - 1.21495*10$

The case where the variable x is as follows; 1.7 <= x < 2.0

FWHM = (0.5λ*XYPSF)/NA

$XYPSF = -0.0078x^3 + 0.0422x^2 - 0.0689x + 1.0478$

The case where the variable x is as follows; 2.0 <= x

FWHM = (0.5λ*XYPSF)/NA

XYPSF = 1.0164

One Point!

The formula of resolution of Z is as follows;

FWHM : Resolution of Z

L : The length of a diagonal line of Square Pinhole

NA = nSINθ :NA of Objective lens

MG : The objective Magnification

N : The refractive index of a medium

Air : 1.0

Water : 1.3

Oil : 1.5

$X = L/(1.22*3.8*\lambda*MG/NA)$

The case where the variable x is as follows; $0 <= x < 2.0$

$FWHM = (2\lambda*ZPSF)/(4n(1-COS\theta))$

$ZPSF = -5.5158*10^{-2}x^5 + 2.0877*10^{-1}x^4 - 2.1172*10^{-1}x^3 + 2.0864*10^{-1}x^2 - 2.2669*10^{-2}x + 6.9038*10^{-1}$

The case where the variable x is as follows; $2.0 <= x$

$FWHM = (2\lambda*ZPSF)/(4n(1-COS\theta))$

$ZPSF = 0.6164x+0.1283$

1-3-2-4 Configuring the filters (When using a spectral detecting system)

When the system incorporates the spectral detector unit that is composed of a 2-channel spectral detector and 1-channel filter, it is possible to set the detection conditions more flexibly, acquire the fluorescence spectral data and use the fluorescence isolation function. As the upper and lower limit wavelengths can be set while acquiring images, any desired wavelength regions can be selected easily.

To acquire image with a spectral detecting system, set the wavelength regions in the [Spectral-Control] sub panel.

Shows the graph of fluorescence wavelength (Em_**) for each dyes. When area inside the graph is double clicked, a window to change graph display method will appear.

Set the upper limit (left) and lower limit (right) wavelengths of the barrier filter.

Move the upper limit and lower limit wavelength of the barrier filter while maintaining the width between the upper limit and lower limit wavelengths.

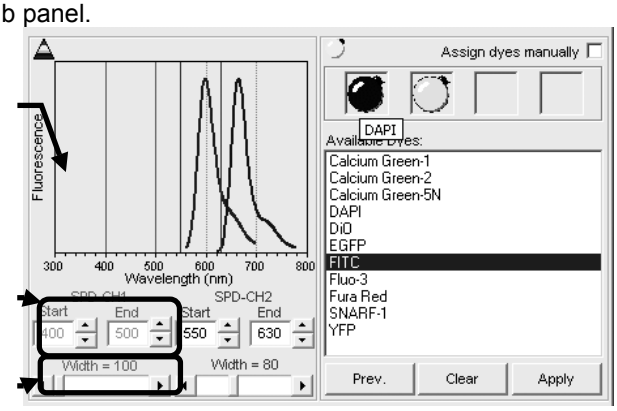
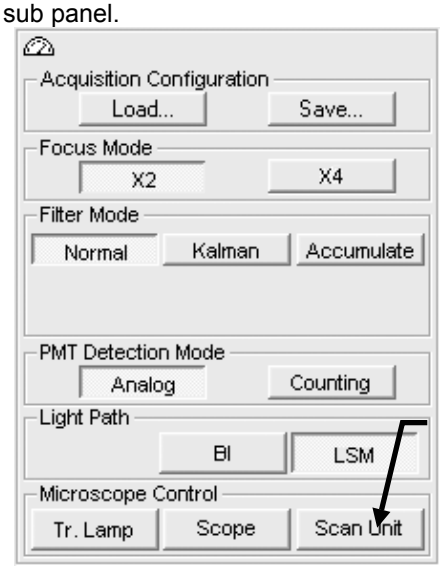


Fig. 1-18 [Spectral-Control] sub panel and [Dyes] sub panel

The settings are interlocked with those in the [Optical System Configuration] window.

The parameters of the spectral detecting system can also be set in the [Optical System Configuration] window as well as in the [Spectral-Control] sub panel.

1. Select the [Settings] sub panel.



<Scan Unit> button
Display the filter and laser types to be set.
(Combination with the laser combiner sets up the laser automatically.)

Fig. 1-19 [Settings] sub panel

2. Click the <Scan Unit> button on the bottom of the [Settings] sub-panel. When the window shown below is displayed, set up the spectral detecting system in the [SPD] group box.

Set the upper limit (left) and lower limit (right) wavelengths of the barrier filter.

Move the upper limit or lower limit wavelength while maintaining the width between the upper limit and lower limit wavelengths.

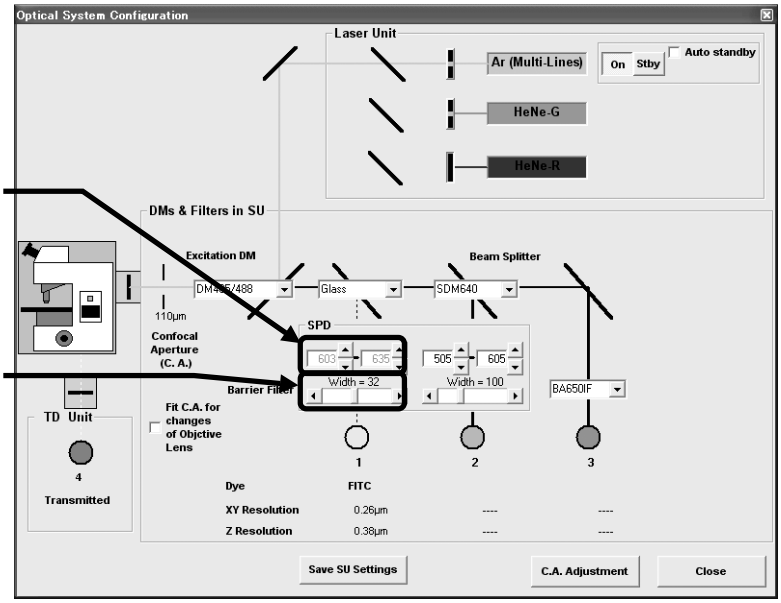


Fig. 1-20 [Optical System Configuration] window

For other functions available in the [Optical System Configuration] window, see section 1-3-2-3, “Configuring the Filters (When using a filter system),”

3. After completing the setup, click the <Close> button to close the window.

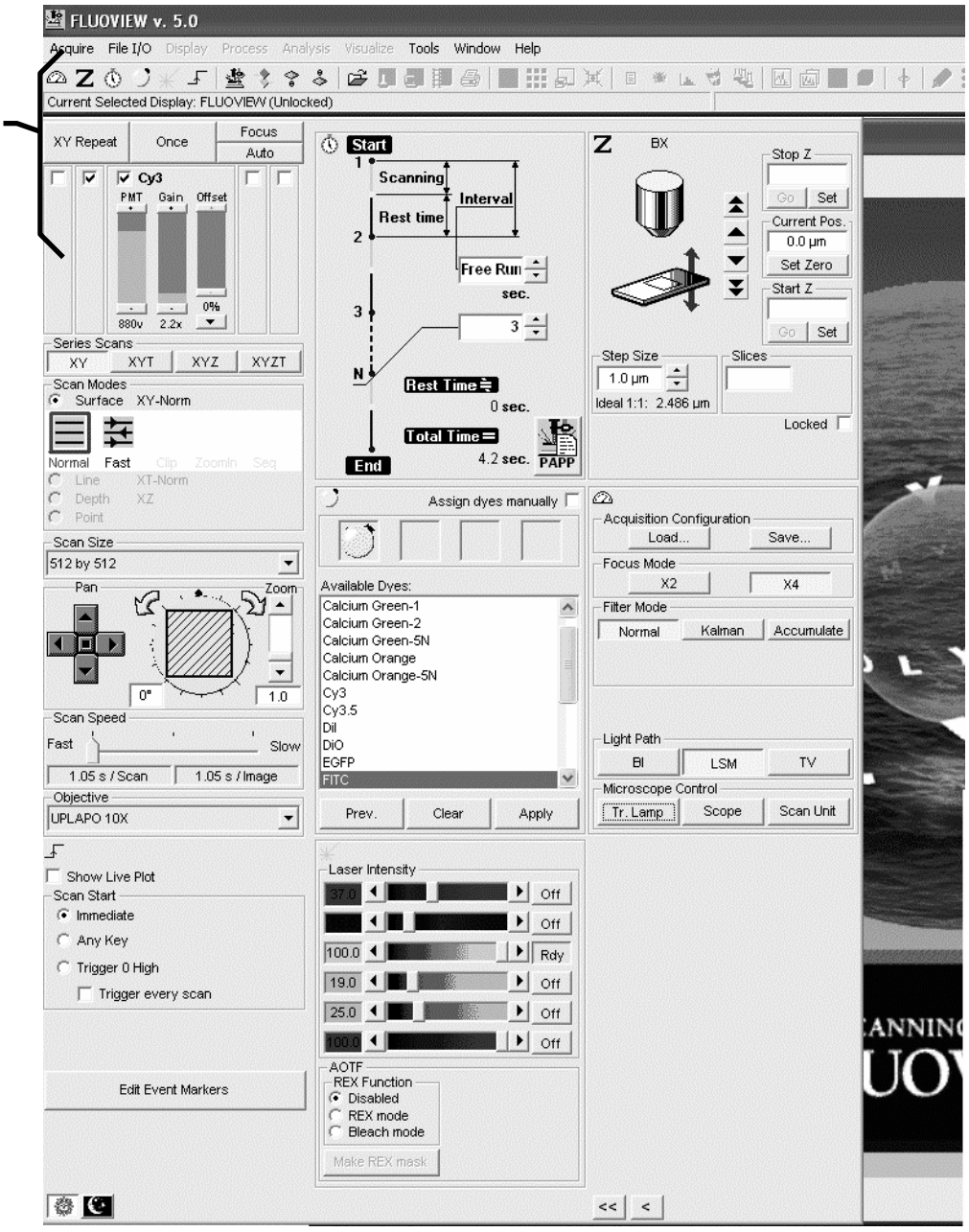
1-3-2-5 Setting the C.A. Diameters

The pinhole diameters are set automatically according to the selected dyeing method.

Use the following procedure to change the pinhole diameters.

1. Display the [Acquire] panel.

[Ch] group box
Sets whether the image of each channel is to be acquired or not, the PMT voltage, Gain and Offset. Right-clicking the mouse inside this group box displays the [Ch] group boxes of all channels.

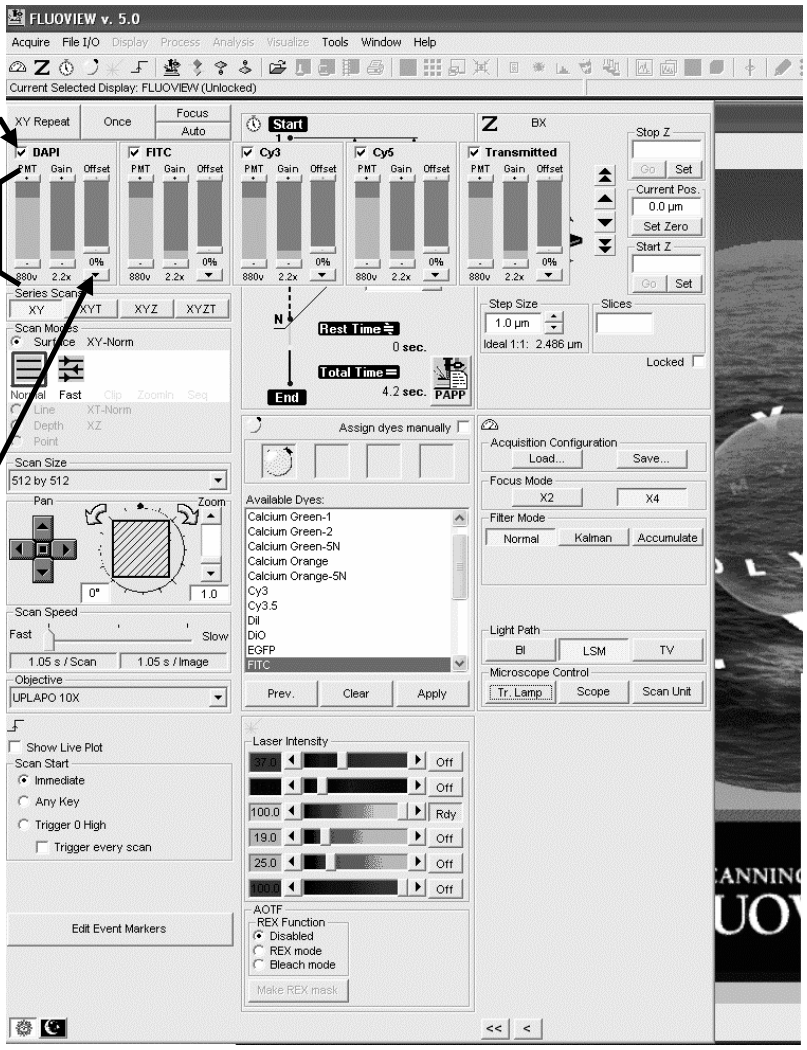


2. Right-click the mouse inside the [Ch] group box to display the [Ch] group boxes of all channels.

[Ch1], [Ch2], [Ch3], [Ch4] and [Ch5] group boxes
Select the channels from which you want to acquire images.

[PMT], [Gain] and [Offset] LED sliders
Set these values independently.
Increasing the PMT Voltage improves the sensitivity.
Increasing the Gain brightens the image.
Increasing the Offset darkens the image.

< ▼ > button
Click to display the pinhole diameter and the laser ND filter setting.



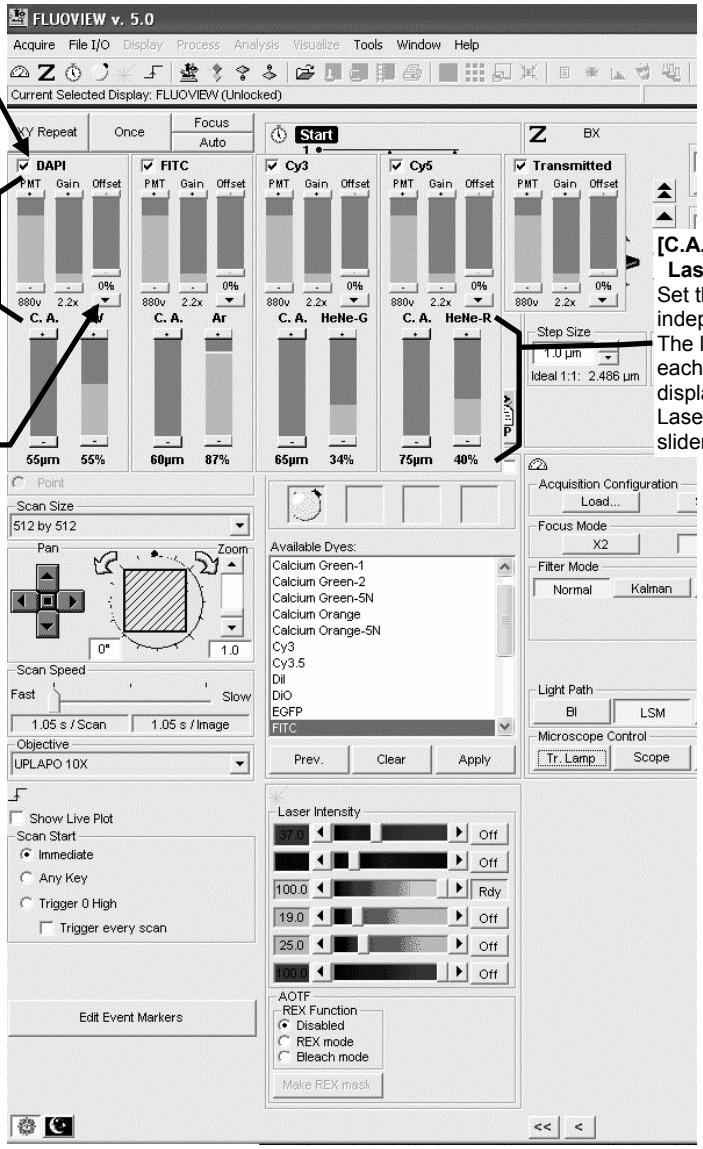
3. Click the < ▼ > button of the channel you want to change the pinhole diameter.

   The group boxes for setting the pinhole diameters and laser ND filter values of the channels are displayed below the [Ch] group box. (They are not displayed for channels set for transmitted light observation.)

[Ch1], [Ch2], [Ch3], [Ch4] and [Ch5] group boxes
Select the channels from which you want to acquire images.

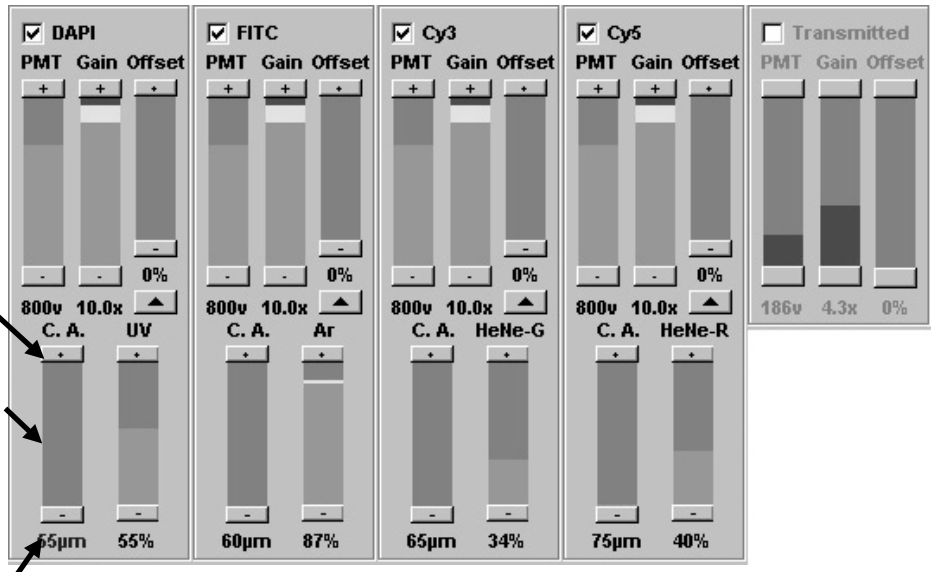
[PMT], [Gain] and [Offset] LED sliders
Set these values independently.
Increasing the PMT Voltage improves the sensitivity.
Increasing the Gain brightens the image.
Increasing the Offset darkens the image.

< ▲ > button
Click to close each group box showing the pinhole diameter and laser ND filter value. The pinhole diameter and laser ND filter value are valid even before the group box is closed by clicking the < ▲ > button.



[C.A.] and Laser ND LED sliders
Set these values independently.
The laser type used with each channel is displayed below its Laser ND LED slider.

4. While observing the images in the [Live] panel, change their pinhole diameter settings.



Clicking this button allows fine adjustment of the value.

Clicking this field allows the value to be changed on a large scale.
The ND value which are usually used are displayed in green.

Clicking the <+> or <-> button or the field displays the set value. The set value can be varied by direct input from the keyboard.

TIP

Each click of the <+> or <-> button of the [C.A.] LED slider increases or decreases the pinhole diameter by 5 nm.

Each click of the slider section of the [C.A.] LED slider varies the pinhole diameter by 25 nm.

Each click of the <+> or <-> button of the Laser ND LED slider increases or decreases the laser ND value by 1%.

Each click of the slider section of the Laser ND LED slider varies the laser ND value by 5%.

TIP

To set to the Confocal Aperture value to its primal value, click the <Apply> button in the [Dyes] sub-panel of the [Acquire] panel.

2 APPLIED OPERATIONS

2-1 General Operation Procedure

This section describes the general image acquisition procedure with the aim to get accustomed with the operation.

Begin using the FLUOVIEW system by acquiring an image or opening an image from a file. For the detailed operation method of each item in the procedure, see the section specified in parentheses (( )).

NOTE **The following is the general operation procedure of FLUOVIEW. Many other functions that are not shown in the following are also available. Please also study their description.**

Turn power ON and start the FLUOVIEW software.
(Sections 1-2-1 & 1-2-2)

(A) **Acquire an image.**
(According to the selected observation mode)

For detailed operation procedures for image acquisition, see section 2-1-1, “Image Acquisition Procedure (Section A)”.

Open an image in a file.
(Section 2-3-2)

Process the image.

Filtering (Section 2-6-1)
Contrast conversion (Section 2-6-2)
Inter-image operation (Section 2-6-3)

Observe the image.

- **Observation of image shape**
  Image display in simulated colors (Section 2-5-1)
  LUT change (Section 2-5-2)
  Simultaneous display of multi-channel images (Section 2-5-4)
  Side-by-side image display (Section 2-5-7)
  Magnified/reduced image display (Section 2-5-9)
  Stereo 3D image display (Section 2-9-3)
  Color-eyeglass 3D image display (Section 2-9-4)
  Animation display (Section 2-9-2)
  Continuous display (Frame-by-frame display) (Section 2-9-1)
- **Observation of image change over time**
  Side-by-side image display (Section 2-10-1)
  Continuous display (Frame-by-frame display) (Section 2-10-2)

Measure the image.

- **Measurement of image shape**
  Length measurement(Section 2-7-3-1)
  Area measurement (Section 2-7-3-2)
- **Measurement of image intensity value and distribution**
  Intensity on a line (Line profile) (Section 2-7-1-1)
  Intensity on a plane (Bird’s eye view) (Section 2-7-1-2)
  Intensity distribution (Section 2-7-2)

Save the image.
(Section 2-3-1)

Compile the presentation data.

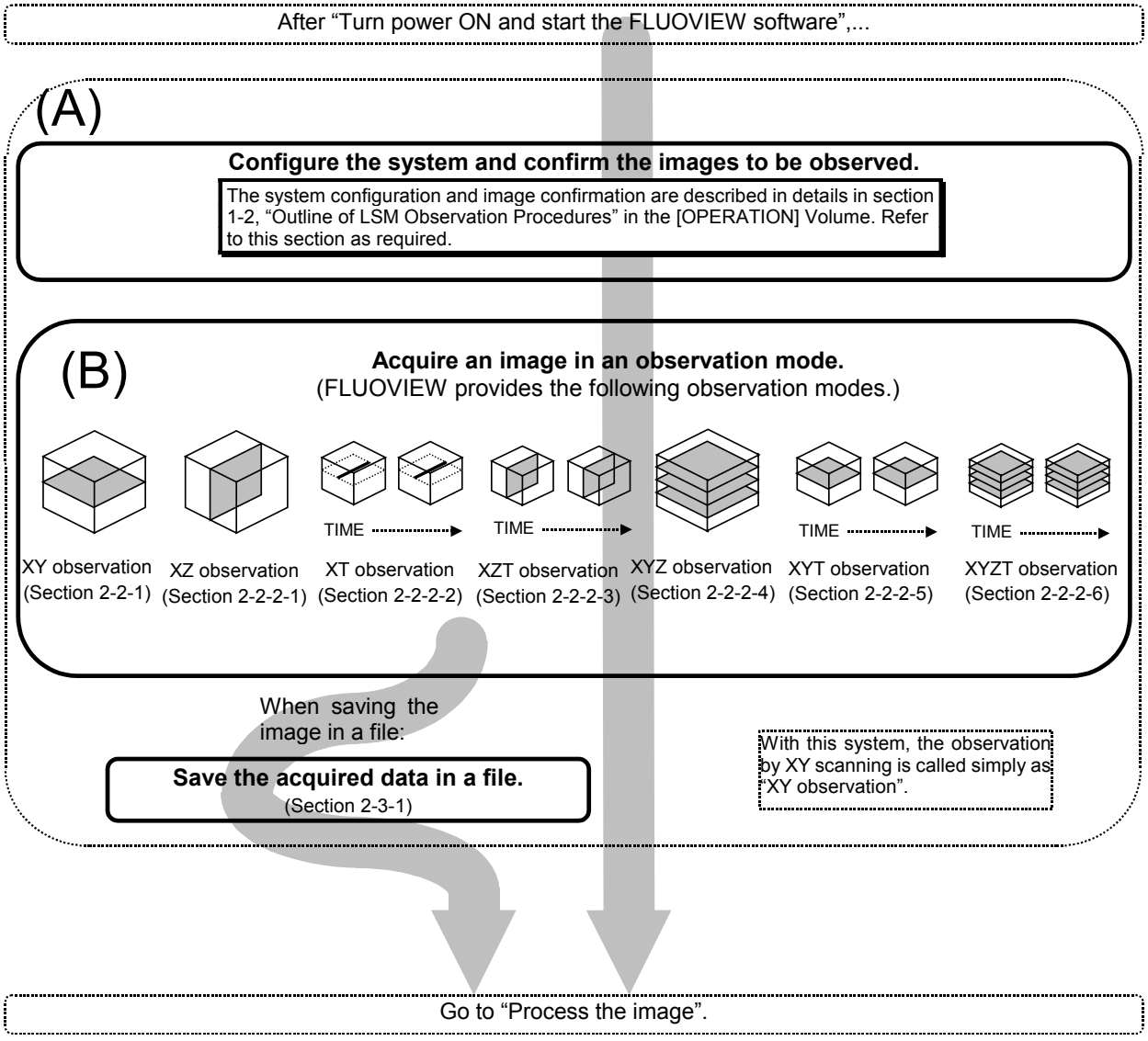
Drawing characters on image (Section 2-12-1)
Drawing pictures on image (Section 2-12-2)
Drawing scales on image (Section 2-12-3)

Output the image at the printer.
(Section 2-13)

Exit from the FLUOVIEW software and turn power OFF.
(Sections 1-2-10)

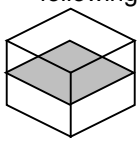
2-1-1 Image Acquisition Procedure (Section (A))

This section describes the procedure for acquiring images. See sections 2-2 and after for the actual operation methods. The detailed operation methods of each item in the procedure are described in the section specified in parentheses (( )).


After “Turn power ON and start the FLUOVIEW software”,...
(A)
Configure the system and confirm the images to be observed.
The system configuration and image confirmation are described in details in section 1-2, “Outline of LSM Observation Procedures” in the [OPERATION] Volume. Refer to this section as required.
(B)
Acquire an image in an observation mode.
(FLUOVIEW provides the following observation modes.)
TIME
TIME
TIME
TIME
XY observation (Section 2-2-1)
XZ observation (Section 2-2-2-1)
XT observation (Section 2-2-2-2)
XZT observation (Section 2-2-2-3)
XYZ observation (Section 2-2-2-4)
XYT observation (Section 2-2-2-5)
XYZT observation (Section 2-2-2-6)
When saving the image in a file:
Save the acquired data in a file.
(Section 2-3-1)
With this system, the observation by XY scanning is called simply as “XY observation”.
Go to “Process the image”.

2-1-2 Image Acquisition Procedure in an Observation Mode (Section (B))

As an example of “Acquire an image in an observation mode”, this section describes the procedure in the XY observation mode. For the procedures in other observation modes, see section 2-2-2, “Image Acquisition in Other Observation Modes” as well as the following procedure.



XY observation
(Section 2-2-1)

After “Configure the system and confirm the images to be observed”...

(B)

Set the acquisition parameters.
(Section 2-2-1-4)

- Observation mode
- Scanning speed
- Image brightness
- Multiple-section to be observed
- Area to be observed

Acquire an image in the XY observation mode.
(Section 2-2-1-5)

When noise is noticeable:

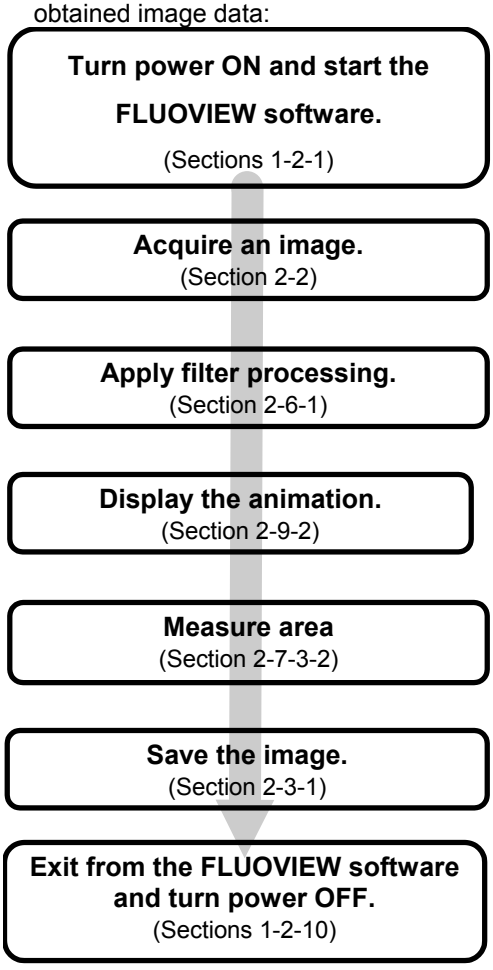
Perform accumulation.
(Section 2-2-1-6)

Go to “Save the acquired data in a file.” or “Process the image”.

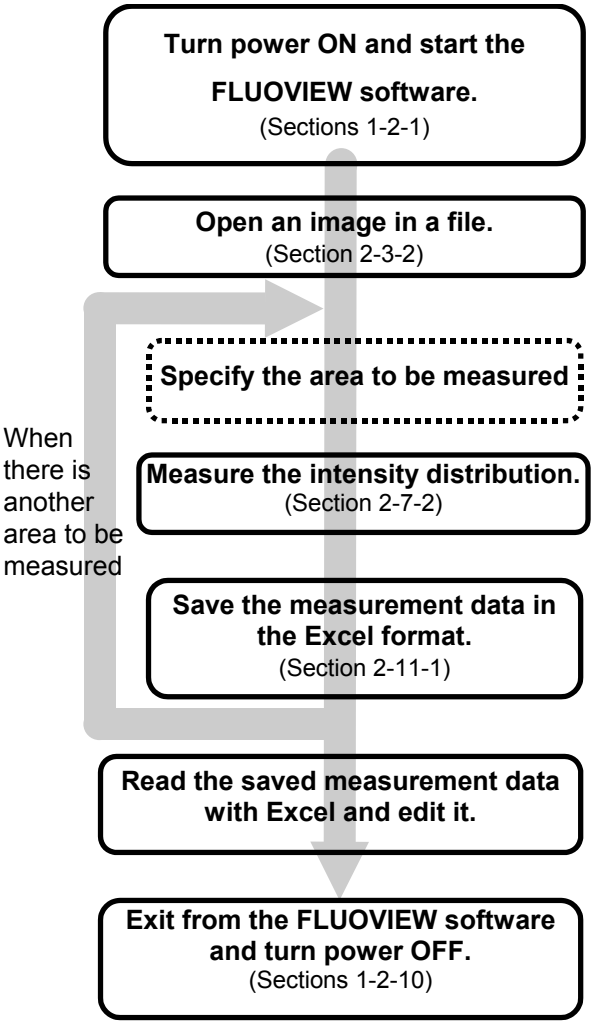
2-1-3 Examples of Operation Procedures

Begin using the FLUOVIEW system by acquiring an image or opening an image in a file. For the detailed operation method of each item in the procedure, see the section specified in parentheses (( )).

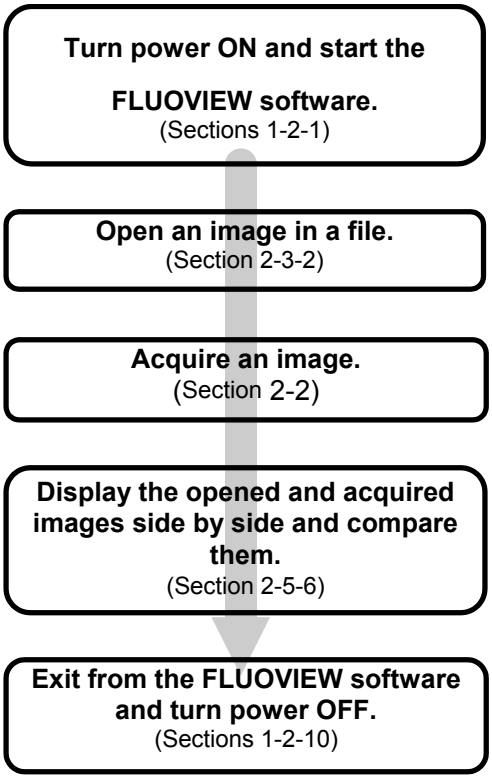
Example 1) To perform XYZ observation of a cell, apply filter processing to the image, display animation to identify the cell shape, then calculate the area, and save the obtained image data:



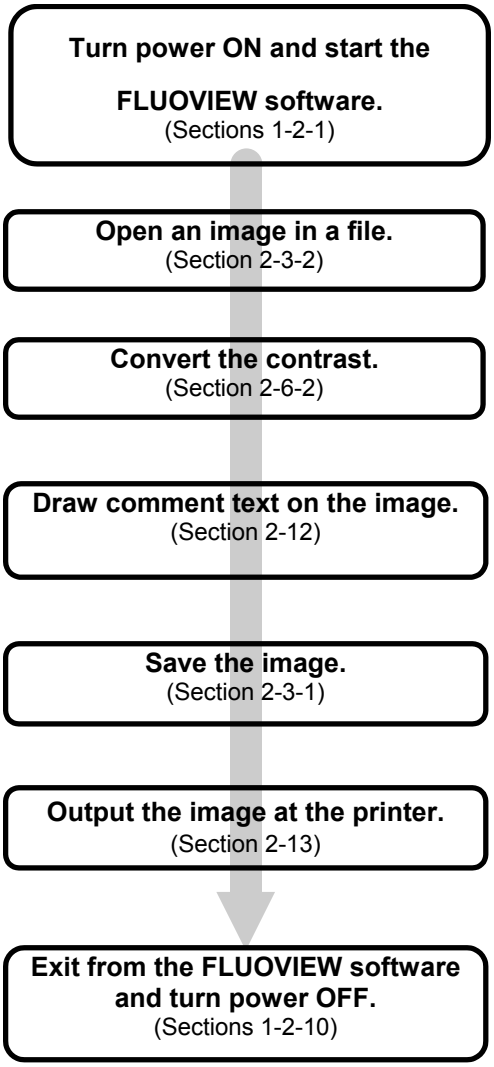
Example 2) To open a previously acquired image in a file, measure the intensity distribution and edit data with Excel:


Turn power ON and start the FLUOVIEW software.
(Sections 1-2-1)
Open an image in a file.
(Section 2-3-2)
Specify the area to be measured
When there is another area to be measured
Measure the intensity distribution.
(Section 2-7-2)
Save the measurement data in the Excel format.
(Section 2-11-1)
Read the saved measurement data with Excel and edit it.
Exit from the FLUOVIEW software and turn power OFF.
(Sections 1-2-10)

Example 3) To acquire an image and compared it with a previously acquired image:


Turn power ON and start the FLUOVIEW software.
(Sections 1-2-1)
Open an image in a file.
(Section 2-3-2)
Acquire an image.
(Section 2-2)
Display the opened and acquired images side by side and compare them.
(Section 2-5-6)
Exit from the FLUOVIEW software and turn power OFF.
(Sections 1-2-10)

Example 4) To open an image in a file, improve its contrast and create a presentation image by entering comment, etc.



2-2 Image Acquisition

Confirm the image to be acquired using the microscope, and acquire its image using FLUOVIEW. The image can be saved in a file as required.

NOTE

If FLUOVIEW window is moved while an image is being acquired, it may cause image acquisition failure.
If at all possible, refrain from moving.

2-2-1 Image Acquisition in XY Observation Mode

This section describes the basic operation procedure from the system configuration to the image acquisition in the XY observation mode and image saving in a file as shown in the following chart. The details of each operation will be described in the subsequent sections.

Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])

Configure the microscope and scan unit.(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])

Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])

Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])

Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])

Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])

Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])

Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])

Adjust the Z-position to observe the desired multiple sections.
(Section 2-2-1-4-7 in [OPERATION])

Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])

Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])

Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])

If the image becomes clean

If the image does not become clean

Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])

Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])

Acquire the image.
(Section 2-2-1-5 in [OPERATION])

Save the image.
(Section 2-3-1 in [OPERATION])

2-2-1-1 Configuring the Microscope

Set the light path so that the image can be observed through the microscope.

1. Display the [Acquire] panel.

<Once> button

Acquires an image in the currently selected observation mode and display the image in the [Live] panel.

<XY Repeat> button

Repeats XY scanning to display images successively in the [Live] panel.

[Ch1], [Ch2] and [Ch3] check boxes
Check to select the image acquisition channel.

[PMT], [Gain] and [Offset] LED sliders
These can be adjusted independently.

Increasing the PMT voltage improves the sensitivity.

Increasing the Gain brightens the image.

Increasing the Offset darkens the image.

Option buttons

Check a button to select one of the displayed observation modes.

[Scan Speed] group box

Sets the scan speed. Sliding to the left of the scale increases the scan speed and to the right decreases it.

Select the magnification of the objective on the microscope.

[Settings], [Z Stage], [Time Series], [Dyes] and [Lasers] sub-panels

These panels are used to set the information required for image acquisition.

<Focus> button

The repetition images can be acquired at a high speed. Use this button to find an optimum image.

The acquired image is displayed.

<Load> button

Loads the observation conditions at image acquisition.

<Save> button

Saves the observation conditions at image acquisition.

[Scan Size] drop down list

Select the image acquisition size.

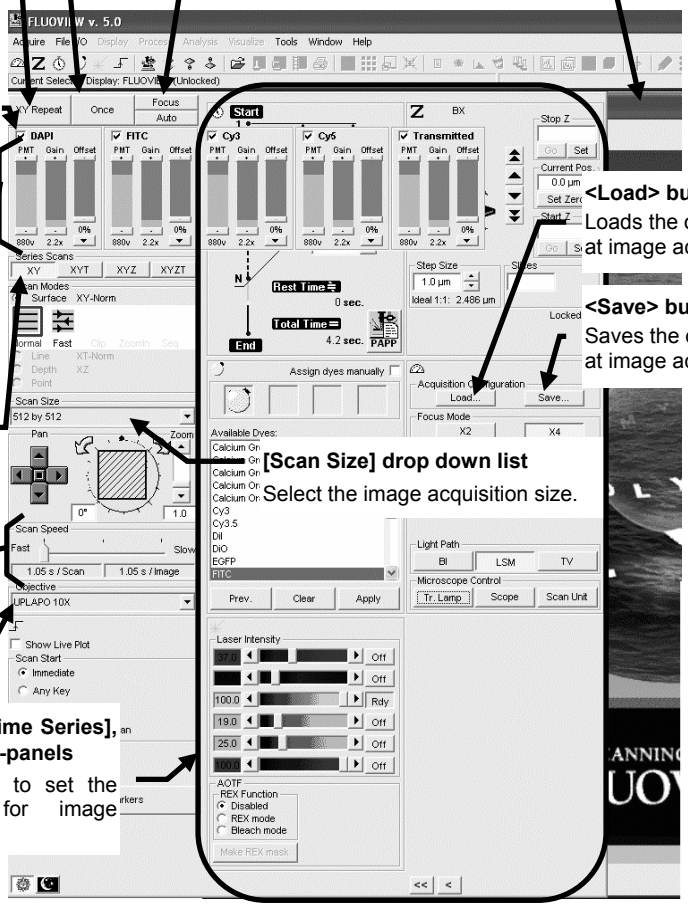


Fig. 2-1 [Acquire] Panel

2. Setting the dyeing method

1) From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

[Available Dyes] list box

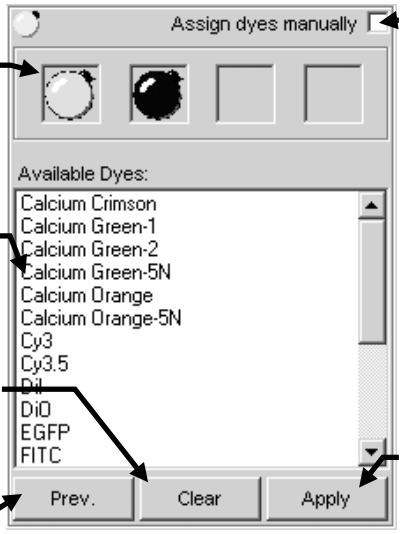
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Clear> button

Clear the set dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.



[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-2 [Dyes] Sub-panel

2) Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

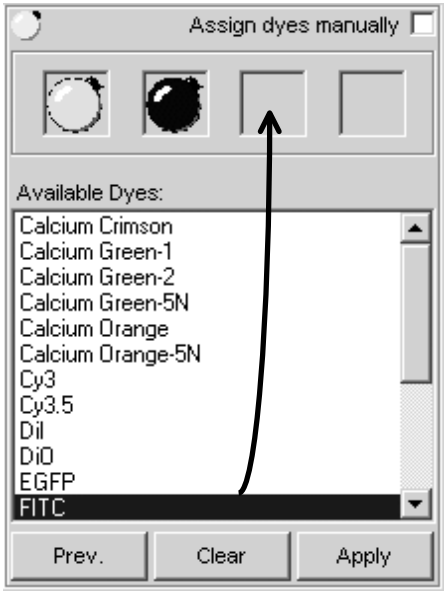


Fig. 2-3 [Dyes] Sub-panel

3) Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

The Confocal Aperture value is also set automatically according to the wavelength and the objective per channels.

TIP

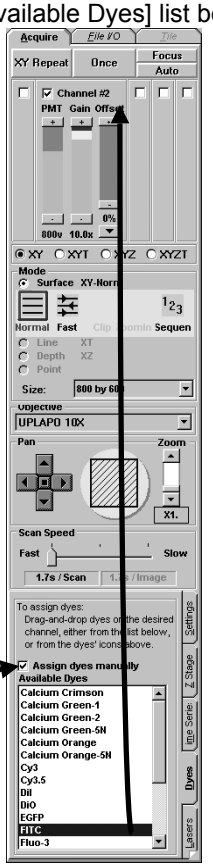
If you switch the objective, click the <Apply> button in the [Dyes] sub panel. The Confocal Aperture value is set appropriately.

For detailes, see section 1-3-2-6 “Setting the Filters” for automatic Confocal Aperture setting with switching the objective.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

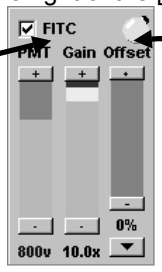
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

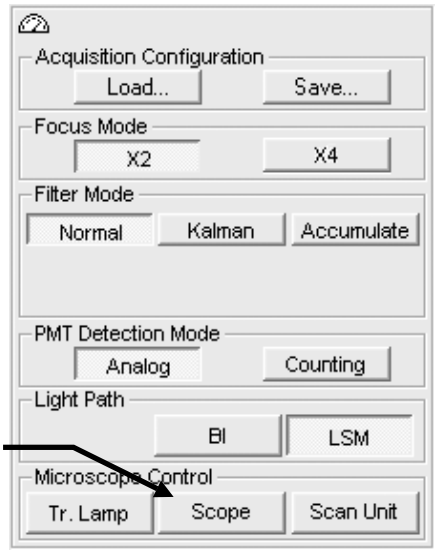
The dyeing method is set



Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

3. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.



<Scope> button

Displays useful information for the system setup.

Fig. 2-4 [Optics] Sub-panel

4. Select the <Scope > button at the bottom of the panel. The window as shown below will appear (in case of a combination with the IX81).

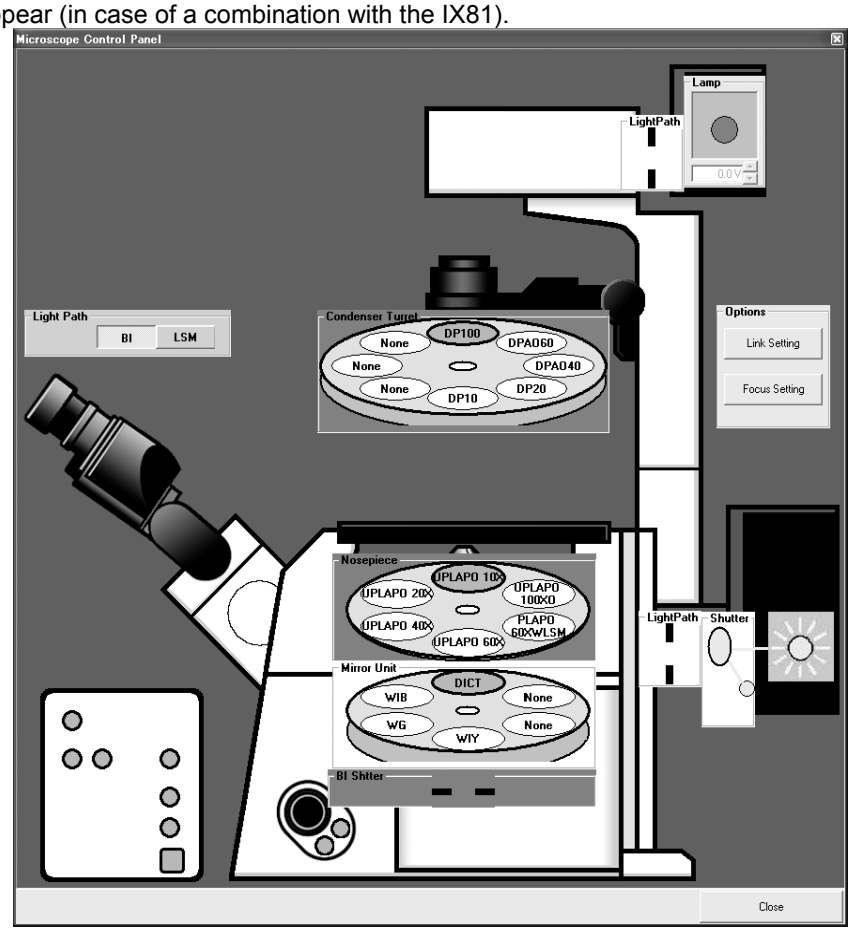


Fig. 2-5 [Microscope Control Panel] Window

5. Configure the microscope in [Microscope Control Panel] window.

6. After completing the setup, click the <Close> button to close the window.

TIP For details of [Microscope Control Panel] window, see section 1-3-2-1 “Configuring the Microscope” in this volume.

7. While looking into the microscope, move the stage and check the observed image.

2-2-1-2 Setting the Filters

The excitation filter, spectral filter and barrier filters are set automatically to the light path according to the dyeing method selected for the specimen.

To change filters, use the following procedure.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

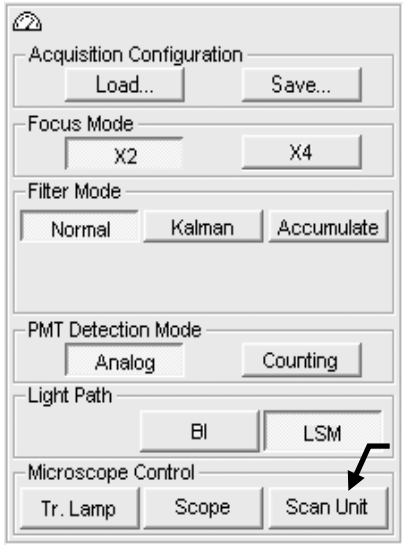


Fig. 2-6 [Settings] Sub-panel

<Scan Unit> button

Click to display the filter and laser types to be set.

(Combination with the laser combiner sets up the laser automatically.)

2. Click the <Scan Unit> button at the bottom of the panel. The window as shown below will appear.

[Laser Unit] group box

Shows the type of laser to be used. (With the laser combiner operation, the laser type is set and displayed automatically.) When the <On> button is pressed-in, the laser is oscillating the beam. When the <Stby> button is pressed-in, the laser is not oscillating. When the [Auto standby] check box is checked, the [After] text box appears below it. When not using laser for a long time, in order to suppress useless electric-power consumption, we recommend you making <Stby> mode. The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser is suspended as standby mode (using Ar or Kr-laser) or the laser oscillation stops(using UV-Ar laser) When the time shown in this box has elapsed after the end of laser scanning.

[Excitation DM] and [Beam splitter] drop-down lists

Change the excitation filter and beam splitter of each channel.

[Fit C.A. for change of Objective Lens] Check box

In case of check this box, Confocal Aperture diameter is set with switching the objective.

[TD Unit] group box

Shows the transmitted light detection.

[Barrier Filter] drop-down list

Changes the barrier filter of each channel.

[Dyes]

Shows the dyeing method set for each channel.

[XY Resolution]

Shows lateral resolution for each channel.

[Z Resolution]

Shows the Z resolution set for each channel.

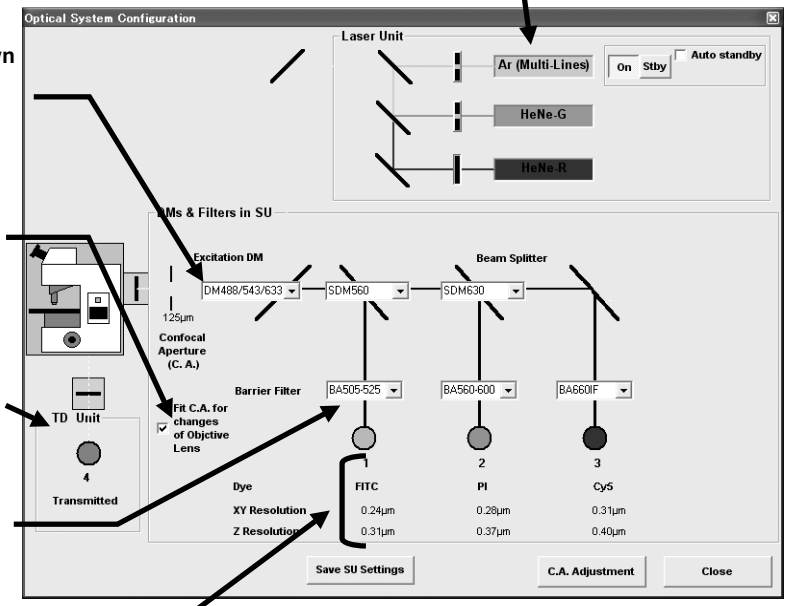


Fig. 2-7 [Optical System Configuration] Window

3. To change the displayed filter types, use the [Excitation DM], [Beam splitter] and/or [Barrier Filter] drop-down lists.

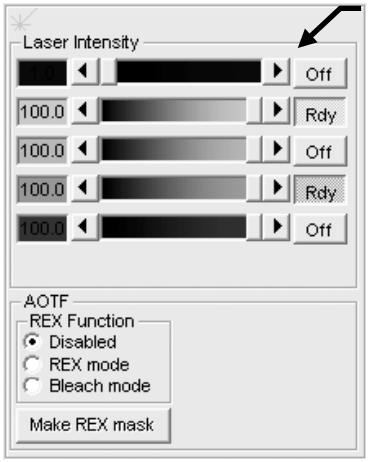
4. After completing the filter setup, click the <Close> button.

2-2-1-3 Setting the ND Filters

When you use the laser combiner, you can set the laser intensity by setting ND filter on the laser combiner.

Display the [Acquire] panel.

Set each laser intensity by sliding the scale bar in the [Laser Intensity] group box of the [Lasers] sub-panel, in accordance with specimen's brightness, fluorescence crosstalk and photo-bleaching.



[Laser Intensity] group box

Set the laser intensity value by the scale bar.

The number of the displayed laser intensity sliders varies depending on that of channels setting for the acquisition.

While using the HeNe green laser, try out the laser power 50% by setting the [Intensity] scale bar in the [Laser Intensity] group box.

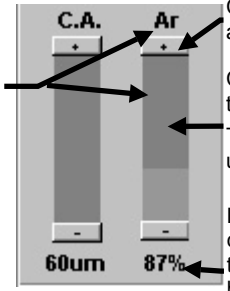
For other lasers, try the laser power 5%.

One Point!

After the dyeing method has been set with the [Dyes] sub-panel, the ND filters can be set using the [Ch] group box in the upper part of the [Acquire] panel.

1. In the upper part of the [Acquire] sub-panel, open the [Ch] group box for the ND to be changed.
2. Click the <More> button.
   The field as shown below is displayed below the [Ch] group box.

Display of the optimum laser and ND value which are set automatically according to the selected dyeing method.



Clicking this button allows fine adjustment of the ND value.

Clicking this field allows the ND value to be changed on a large scale.

The ND values which are usually used are displayed in green.

Display of the ND value set by clicking the <+> and <-> buttons or the field. The set ND value can also be changed by entering its value directly from the keyboard.

3. Vary the ND value using the Laser LED slider.
   Each click of the laser ND <+> or <-> varies the laser ND value by 1%.
   Each click of the laser ND slider varies the laser ND value by 5%.

2-2-1-4 Setting the Observation Condition

1 Setting the Objective Magnification

1. From the drop-down list on the center of the [Acquire] panel, select the objective being used with the microscope.

NOTE **The measurement results will be inaccurate if the objective magnification set here does not match the actual magnification of the objective in use.**

TIP If you change the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.
For details, see section 1-3-2-6 “Setting the Filters” for automatic Confocal Aperture setting with switching the objective.

2 Setting the Zoom Ratio to 1X

1. Use the [Zoom] scale in the [Acquire] panel to set the zoom ratio to “X1”.

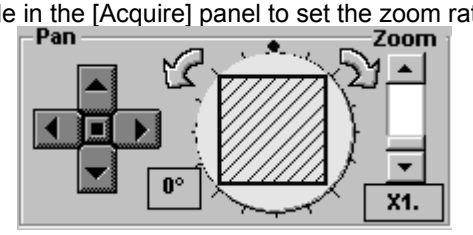
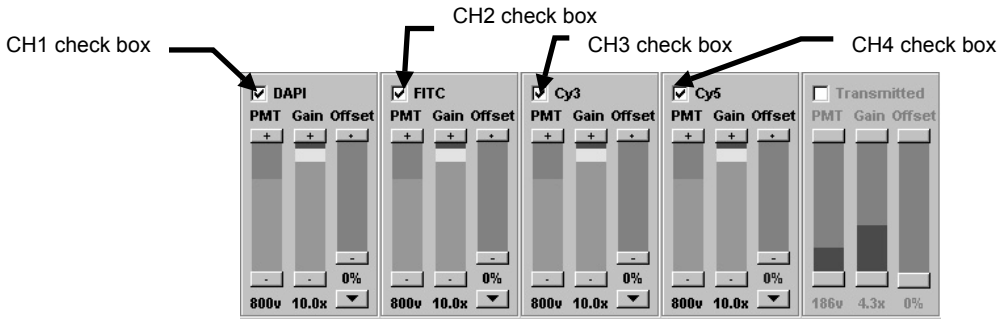


Fig. 2-8 [Pan]/[Zoom] Group Box

Using the UV-Ar laser, set the zoom ratio to “X2”.

3 Setting the Channels

1. In the Channel 1 group box, check the check box showing the applicable dyeing method to make the image acquisition ready.
2. In the Channel 2 group box, check the check box showing the applicable dyeing method to ready the image acquisition.
3. In the Channel 3 group box, check the check box showing the applicable dyeing method to ready the image acquisition.
4. In the Channel 4 group box, check the check box showing the applicable dyeing method to ready the image acquisition.



TIP

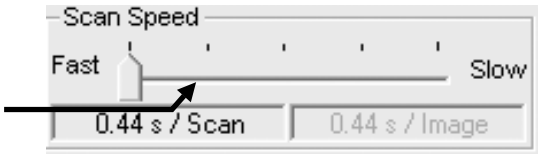
To display the information on all channels simultaneously, right-click the boundary between channel display boxes.
Click the boundary again to return to the original display.

4 Setting the Highest Scan Speed

Set the scan speed to the fastest speed by using the scale in the [Scan Speed] group box in the [Acquire] panel

[Scan Speed] group box

Set the scan speed by clicking a point on the scale line.



TIP

The focus mode makes it possible to increase the line skipped scan speed.

From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

Select either option button in the [Focus Mode] group box.

[Focus Mode] group box

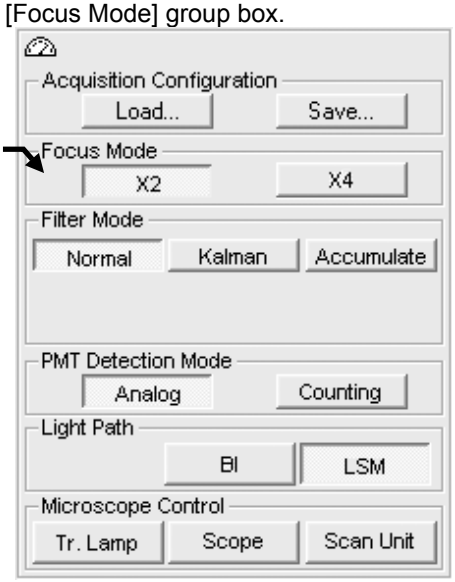
[X2] option button

Acquires image at twice the highest speed.

[X4] option button

Acquires image at 4 times the highest speed.

Increasing the number of divided images in the image window, line skipped scan at 4 times (Focus) cannot be done.



The focus mode is enabled when acquiring images using the <Focus> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

5 Setting the XY Observation Mode

1. In the [Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Acquire] panel, select the XY observation mode option button.

6 Repeated Scanning Operation

<XY Repeat> button

1. Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel.

<Focus> button

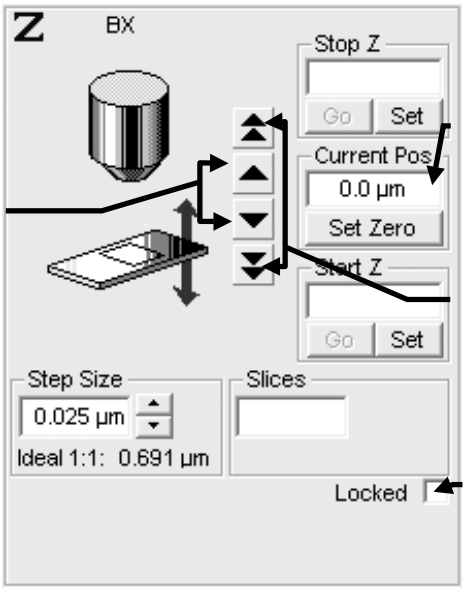
TIP

Use the <FOCUS> button to acquire image at an even higher speed. If the specimen is already being scanned, stop scanning with the <STOP SCAN> button before selecting the <XY Repeat> button.
The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

7 Setting the Multiple sections to be Observed

While acquiring image, move the Z stage to select the multiple sections to be observed.

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



<Z stage fine adjustment> buttons
Displace the Z stage by 0.1 µm per step.

[Current Pos] text box
Shows the current position of the stage. The value can also be entered directly from the keyboard.

<Z stage coarse adjustment> buttons
Displace the Z stage by 1.0 µm per step.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-9 [Z Stage] Sub-panel

TIP

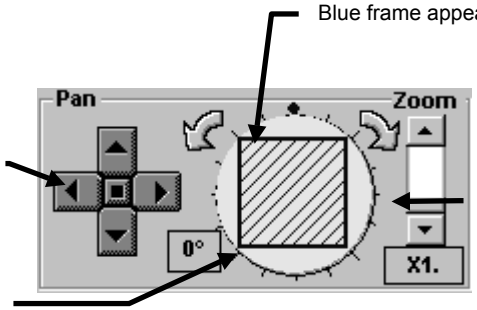
The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

2. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

8 Setting the Area to be Observed

When the observation targets are concentrated in a narrow area or when observation of a specific area detail is required, the image of a limited area can be selected.



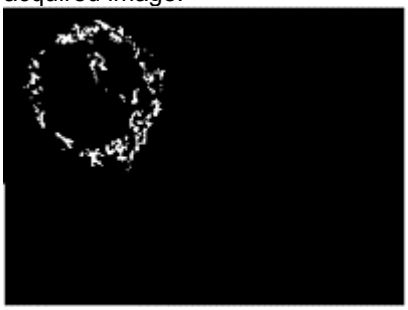
The 4 buttons represent directions, and clicking a button moves the acquired image area in the direction indicated by the button.
Clicking the square button on the center returns the acquired image area to the center.

Click a point inside the circle to change the position of the acquired image area directly.

Clicking a point in the scale area to change the value on a large scale.
Clicking the top or bottom arrow button allows fine adjustment of the value.
Dragging the square knob allows the value to be changed directly.

Fig. 2-10 [Pan]/[Zoom] Group Box

For instance, let us assume that the observation target is deviated at the top left of the acquired image.



With this example, the area containing the observation target can be observed using the following procedure.

1. Increase the zoom ratio using the [Zoom] scale in the [Acquire] panel.

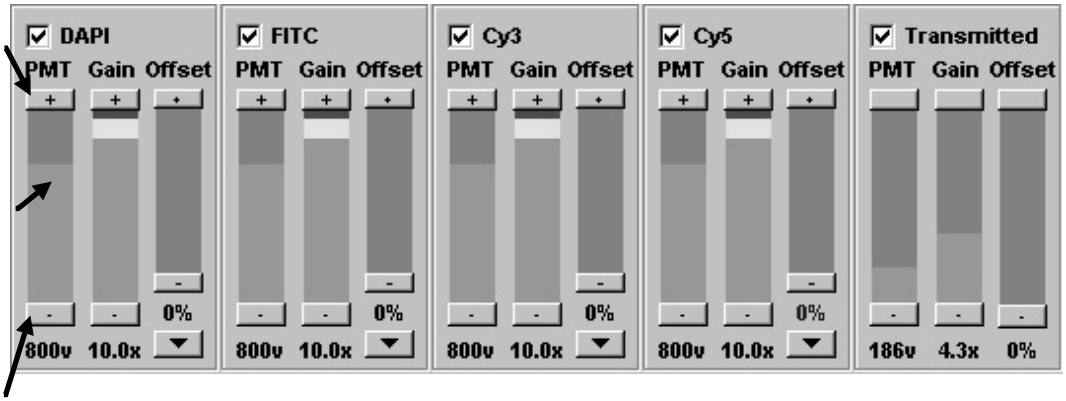
2. The light-blue circle to the left of the [Zoom] scale represents the field visible through the microscope, and the blue frame indicates the acquired image area. Move the blue frame inside the circle using the <Pan> buttons so that the desired observation targets are displayed in the [Live] panel.

9 Adjusting the image brightness

Clicking this button allows fine adjustment of the value.

Clicking this field allows the value to be changed on a large scale.
The ND value which are usually used are displayed in green.

Clicking the <+> or <-> button or the field displays the set value. The set value can be varied by direct input from the keyboard.



1. While observing the image in the [Live] panel, change the setting values of the **PMT voltage**[PMT], **Offset**[Offset] and **Gain**[Gain] in the [Acquire] panel. The functions of these parameters are as described below.

PMT Voltage	Increasing this value improves the sensitivity. However, too high a sensitivity makes image noise noticeable. If sufficient brightness cannot be obtained by setting the PMT voltage to 800 V, leave it as it is and increase Gain. This will usually provide a better result than using a PMT voltage over 800 V.
Offset	Darkens the image at the ratio set during image acquisition. (This value can be set independently from the Gain.)
Gain	Brightens the image at the ratio set during image acquisition. (This value can be set independently from the Offset.)

For example...

When the acquired image is dark or the observation targets are hardly visible, increase **PMT voltage** to improve the sensitivity. If the image resulting from this becomes too bright, first try decreasing **PMT voltage** slightly. If the image background is still too bright, increase **Offset** to darken the background. As this also reduces the brightness of the observation target, increase **Gain** as required so that the observation target is clearly visible.
Gamma Correction provides you more brightened image even if it was acquired with darkness.
See “2-5-2-2 LUT Graph Editing by Gamma Correction “ for details in this operation.

TIP

Each click of the <+> or <-> button of the [PMT] LED slider increases or decreases the PMT voltage by 5 V.

Each click of the slider section of the [PMT] LED slider varies the PMT voltage by 25 V.

In the transmitted channel case, each click of the <+> or <-> button of the [PMT] LED slider increases or decreases the PMT voltage by 1V.

In the transmitted channel case, each click of the slider section of the [PMT] LED slider varies the PMT voltage by 10V.

Each click of the <+> or <-> button of the [Gain] LED slider increases or decreases the Gain by X0.1.

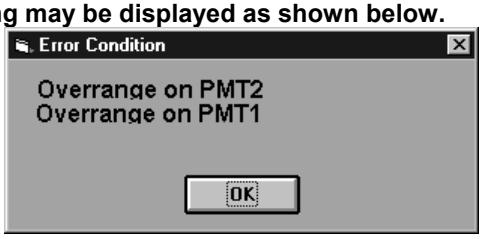
Each click of the slider section of the [Gain] LED slider varies the Gain by .X1.0.

Each click of the <+> or <-> button of the [Offset] LED slider increases or decreases Offset by 1%.

Each click of the slider section of the [Offset] LED slider varies the Offset by 5%.

NOTE

Photomultiplier is used for sensor. When the light incident to photomultiplier tube is too bright or PMT voltage is set to a high voltage, PMT Over warning may be displayed as shown below.



This warning is displayed to protect photomultiplier tube when the light incident to it exceeds a certain level. When it is displayed, decrease PMT voltage setting.

<LUT> button

[Hi-Lo]LUT

TIP

[Hi-Lo] LUT can be utilized to adjust image intensity easily.

1. Click <LUT> button from tool bar. Dialog box – [Color Tool] will appear.
2. Click [Hi-Lo] LUT from group box of [Standard Color LUTs].
3. The intensity value 0 is colored with Blue, and the maximum intensity is colored with Red.
4. When there is noises in the image, different colors may appear in its background. When the intensity is saturated, the portion will be colored with Red. Based on this coloring, you may adjust the image intensity.

TIP

[Hi-Lo] LUT switching can be done by use of Hot key even if during image acquisition.

The image channel can be assigned in [Acquire] panel

The LUT of the assigned channel can be switched to [Hi-Lo] LUT by pressing Ctrl + H. By pressing Ctrl + H again, LUT returns to the previous. The LUT of all channels can be switched to [Hi-Lo] LUT by pressing Ctrl + Shift + H.

10 Setting a Lower Scan Speed

1. The scan speed can be decreased using the scale in the [Scan Speed] group box on the [Acquire] panel.

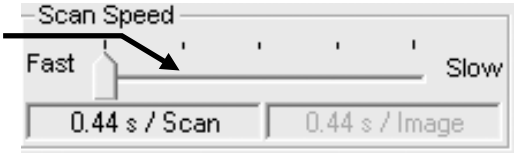
TIP

In general, setting a lower scan speed allows the acquired image quality to be improved.
However, a low scan speed also lengthens the time required for image acquisition.

NOTE

When the scan speed is decreased during fluorescence observation, the saturation of fluorescence may darken the image of certain types of specimens. In this case, increase the scan speed and increase the PMT Voltage or use accumulation in scanning.

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



11 Stopping Repeated Scanning

1. After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily.

2-2-1-5 Acquiring Image

<Once> button

1. Select the <Once> button. The acquired image will be displayed in the [Live] panel.

2-2-1-6 Acquiring Image in Accumulation Mode

When the image is dark or noisy, use an accumulation mode in image acquisition to improve the image quality.

Kalman Accumulation and Peak Accumulation

- The Kalman accumulation acquires images for the specified number of times while averaging the images. This operation is effective for reduction of noise.
- The Peak accumulation acquires images for the specified number of times while adding the images, and stops image acquisition when any intensity value on the image reaches the peak (4095). This operation is effective for acquiring an image with the dark lower part in the XYZ observation and observation of an extremely dark image.

Kalman Accumulation Algorithm

Every time an image is acquired, the pixel values are rewritten based on the following formulae, where it is assumed that;

n: number of image acquisitions;
I(n): Result of n-times of Kalman accumulations (Intensity values of pixels);
I(new): New intensity value obtained after every image acquisition.

The result of the first Kalman accumulation is identical to the result of ordinary image acquisition:
I(1) = I(new)
The result of the n-th (n > 1) Kalman accumulation is:
I(n) = (I(n-1) * (n+1) + I(new)) / n

1 Acquiring Image in Accumulation Mode (Frame mode)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

[Filter Mode] group box
Select the accumulation mode.
Two accumulation modes, [Kalman] and [Accumulate To Peak] are available.

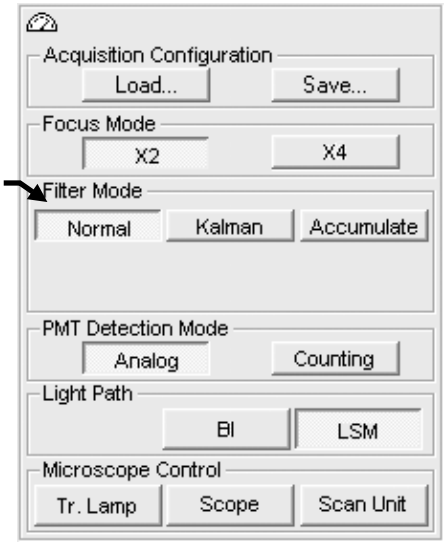
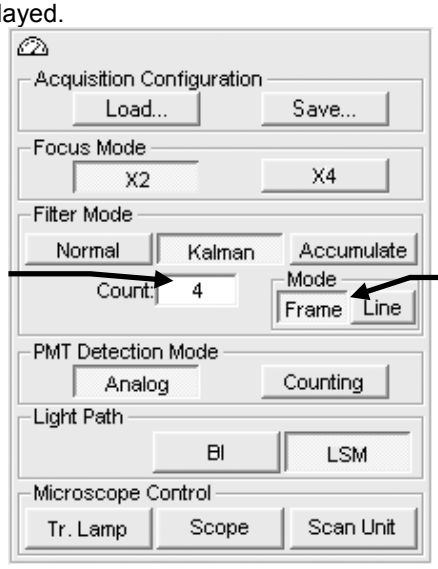


Fig. 2-11 [Settings] Sub-panel

2. In the [Filter Mode] group box, select the [Kalman] or [Accumulate] option button.
3. When [Kalman] is selected, enter the accumulation count in the text box. And select the <Frame> button displayed.

Enter the accumulation count.



<Frame> button

TIP

The accumulation count can be set up a maximum of 64 times.
When 0 is set as the number of times of accumulation, the number is set to 1 automatically.

4. When [Accumulate] is selected, enter the addition count in the text box.

5. Click the <Once> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

2 Acquiring Image in Accumulation Mode (Line mode)

NOTE **Image acquisition in the line mode can be performed when you use the FV1000 system with AOTF (FV5-COMBA).**

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel.

[Filter Mode] group box
Select the accumulation mode.
Two accumulation modes, [Kalman] and [Accumulate To Peak] are available.

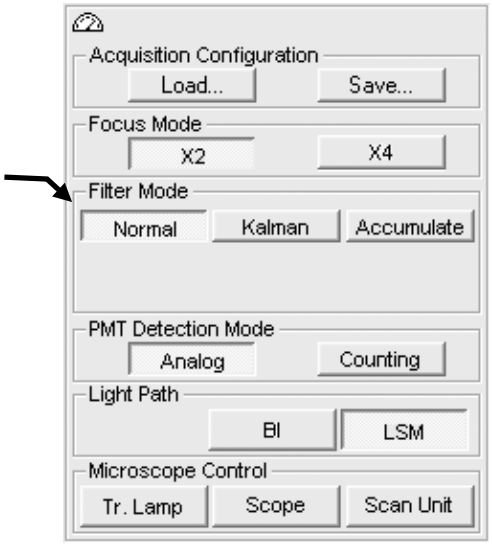
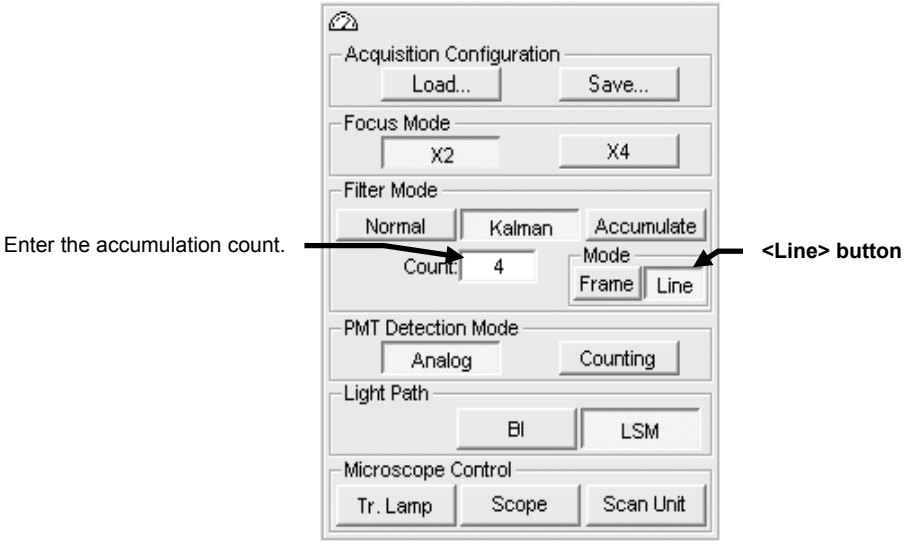


Fig. 2-12 [Scan] sub-panel

2. In the [Filter Mode] group box, select the [Kalman] option button.

3. Enter the accumulation count in the text box and select the <Line> button displayed.



TIP

The accumulation count can be set up a maximum of 64 times.

When 0 is set as the number of times of accumulation, the number is set to 1 automatically ..

4. Click the <Once> button in the [Acquire] panel.

   The acquired image will be displayed in the [Live] panel.

   And the accumulated image can be displayed in the image window without being displayed in full.

The difference between the Frame and Line modes of Kalman Accumulation

In frame mode, the image is accumulated every one frame during acquisition.

In line mode, accumulation is done every one line during acquisition.

The frame mode is intended to utilize for fixed specimen observation, and the line mode is for living specimen observation. Line mode makes it possible to shorten sampling interval so that it is suitable for live cell observation.

2-2-1-7 Saving the Acquired Image in File

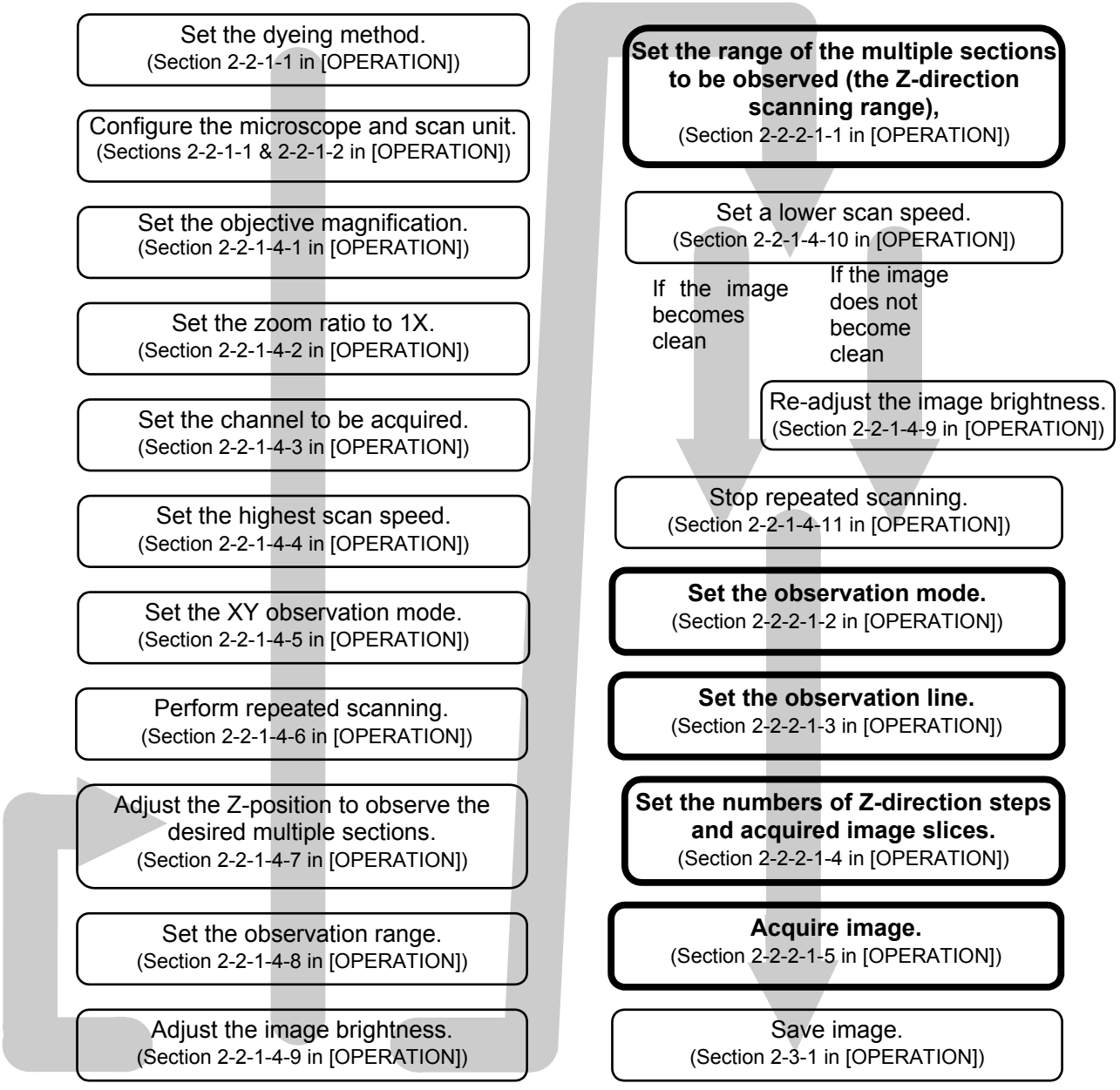
<Experiment> button

1. Display the [File I/O] panel.
2. Click the page tab of the [Live] panel showing the image to be saved, so that the image is displayed at the front.
3. Click the <Experiment> button in the [Save] group box in the [File I/O] panel.
   For details, see section 2-3-1, “Saving Image”.

2-2-2 Image Acquisition in Other Observation Modes

2-2-2-1 XZ Observation Mode

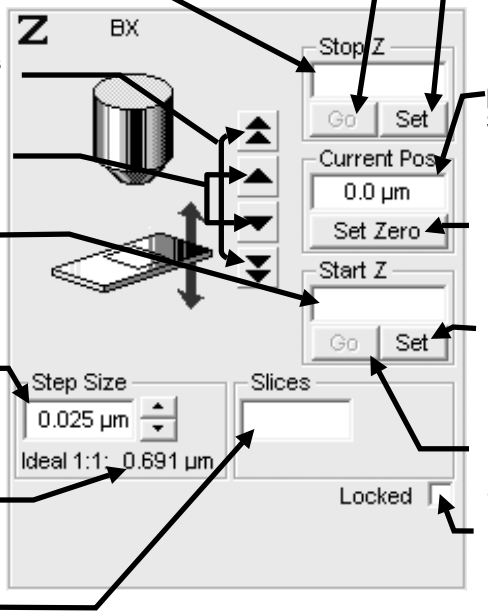
The description in this section will be focused on the image acquisition operations in the XZ observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired multiple sections.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the multiple sections to be observed (the Z-direction scanning range),
(Section 2-2-2-1-1 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-1-2 in [OPERATION])
Set the observation line.
(Section 2-2-2-1-3 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-1-4 in [OPERATION])
Acquire image.
(Section 2-2-2-1-5 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (i.e. the Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-13 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).

4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the observation mode

1. In the [Mode] group box in the [Acquire] panel, select the [Depth] option button.

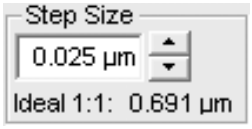
2. In the [Acquire] panel, select the XZ observation mode option button.

3 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

4 Setting the numbers of steps and acquired image slices

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



[Step Size] text box

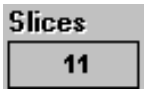
2. The number of steps has already been set and displayed in the [Step Size] text box. This number can be changed using the <▲> or <▼> button in the [Step Size] text box.

TIP

The number of steps in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.

NOTE

The number of steps calculated by the system may be erroneous unless the XZ observation mode has been set previously.



[Slices] text box

TIP

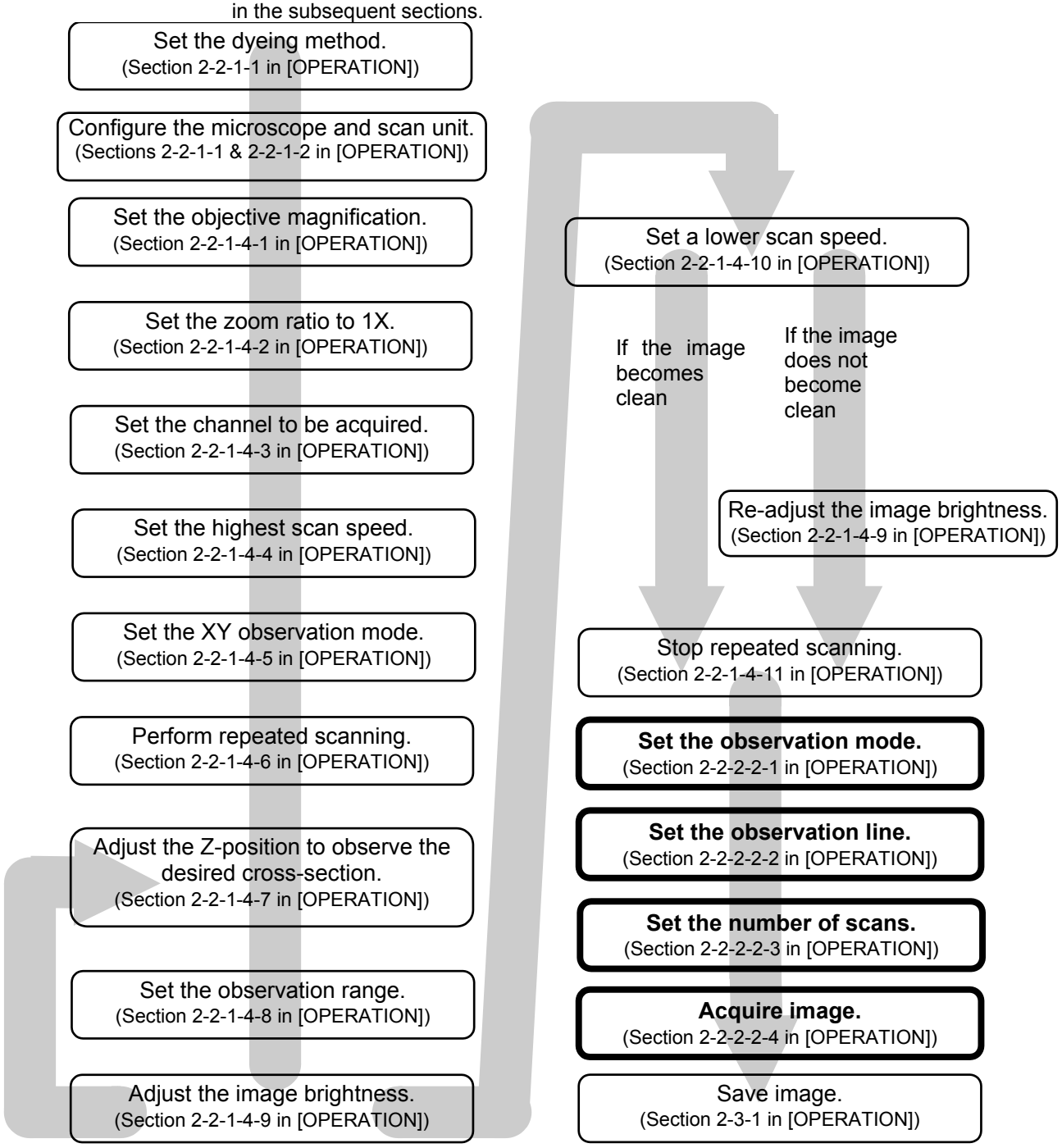
The number of acquired images shown in the [Slices] text box can also be input from the keyboard.
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

5 Acquiring image

1. Click the <XZ> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

2-2-2-2 XT Observation Mode

The description in this section will be focused on the image acquisition operations in the XT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-2-1 in [OPERATION])
Set the observation line.
(Section 2-2-2-2-2 in [OPERATION])
Set the number of scans.
(Section 2-2-2-2-3 in [OPERATION])
Acquire image.
(Section 2-2-2-2-4 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the observation mode

1. In the [Mode] group box in the [Acquire] panel, select the [Line] option button.
2. In the [Acquire] panel, select the XT observation mode option button.

2 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

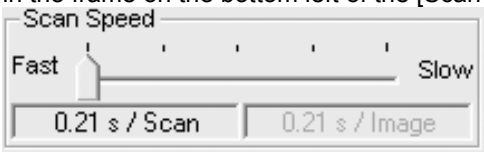
3 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

4 Acquiring image

1. Click the <XT> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

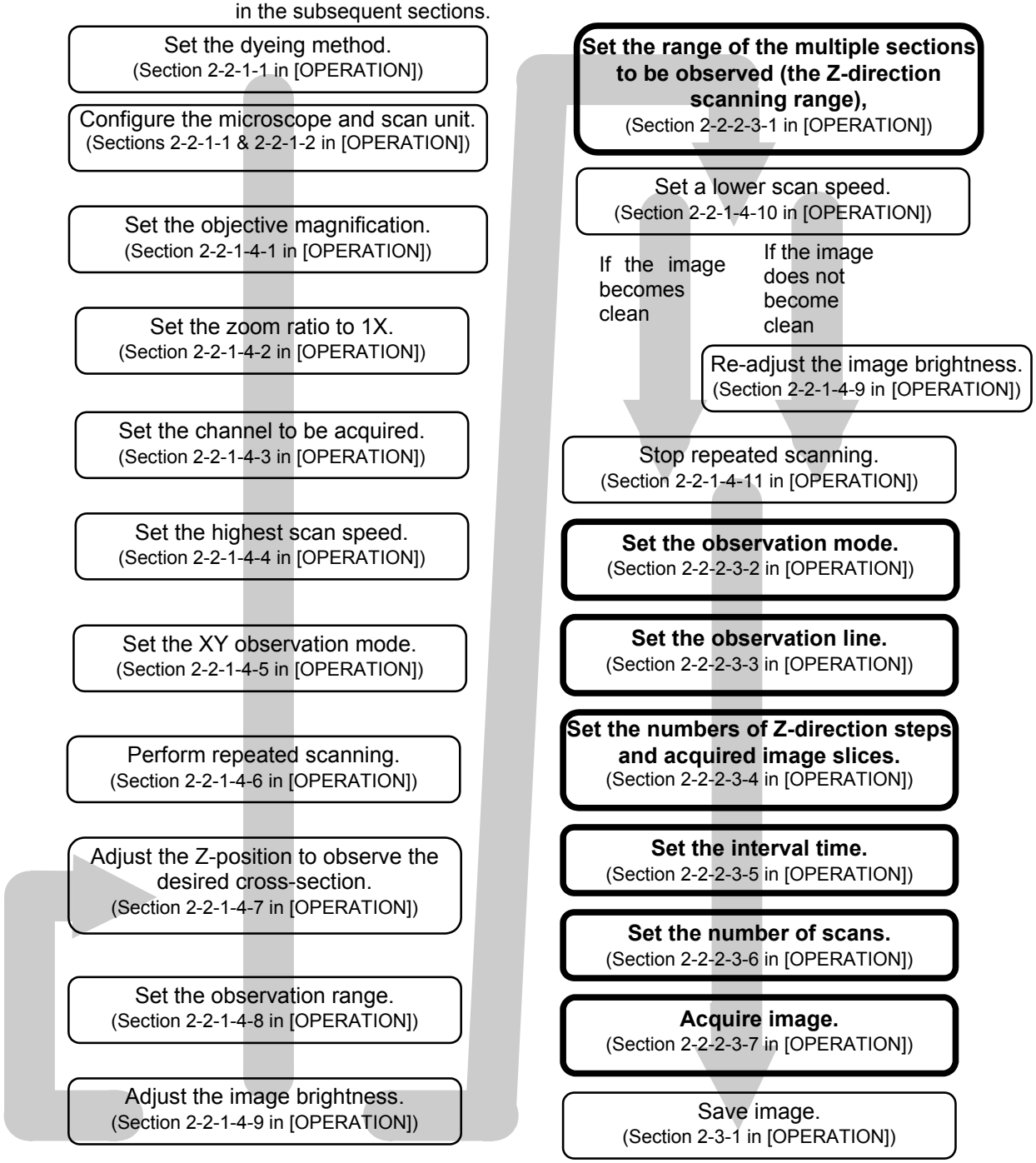
The XT observation acquires the images of the same position (line) for 2000 times successively at the set scanning speed. For reference, the time required for the image acquisitions is shown in the frame on the bottom left of the [Scan Speed] group box.



The number of image acquisitions (2000 times) can be changed by changing the value in the [N] text box in the [Time Series] sub-panel.

2-2-2-3 XZT Observation Mode

The description in this section will be focused on the image acquisition operations in the XZT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the multiple sections to be observed (the Z-direction scanning range),
(Section 2-2-2-3-1 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-3-2 in [OPERATION])
Set the observation line.
(Section 2-2-2-3-3 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-3-4 in [OPERATION])
Set the interval time.
(Section 2-2-2-3-5 in [OPERATION])
Set the number of scans.
(Section 2-2-2-3-6 in [OPERATION])
Acquire image.
(Section 2-2-2-3-7 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

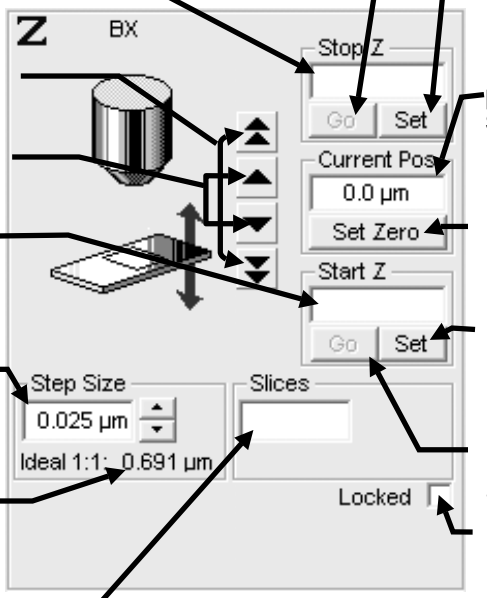
<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.



<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-14 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).

4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the observation mode

1. In the [Mode] group box in the [Acquire] panel, select the [Depth] option button.

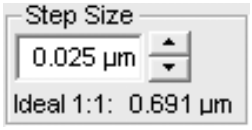
2. In the [Acquire] panel, select the XZT observation mode option button.

3 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

4 Setting the numbers of steps and acquired image slices

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

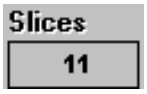


[Step Size] text box

2. A number of steps is displayed in the [Step Size] text box. This number can be changed using the <▲> or <▼> button in the [Step Size] text box.

TIP

The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



[Slices] text box

NOTE

The number of steps calculated by the system may be erroneous unless the XZT observation mode has been set previously.

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

5 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel.

2. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

[Interval] text box
Set the interval time using the <▲> or <▼> button or by input from the keyboard.

[N] text box
Set the number of scans using the <▲> or <▼> button or by input from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

[Rest Time] text box
Shows the time after end of acquisition of an image until the start of next acquisition.

[Total Time] text box
Shows the total time required for image acquisitions.

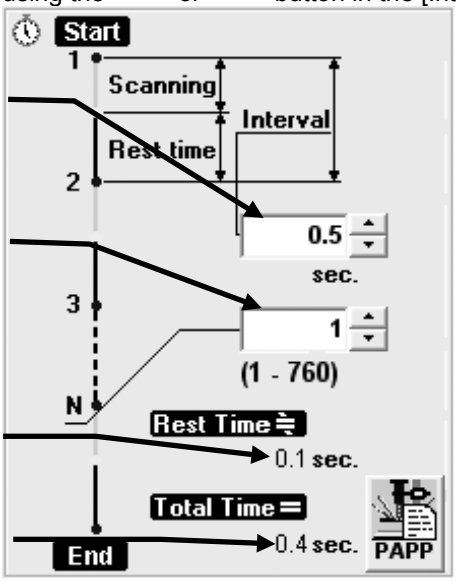


Fig. 2-15 [Time Series] Sub-panel

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.

In this case, the [Interval] text box shows “Free Run” message.

Using <▼> button also sets the [Interval] text box to “Free Run”.

6 Setting the number of scans

2. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

7 Acquiring image

1. Click the <XZT> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

While acquiring an image in the XZT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Resume> button
Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

8 Appending image

XZT image can be added after the image acquisition.
Immediately after acquisition of an image in the XZT observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

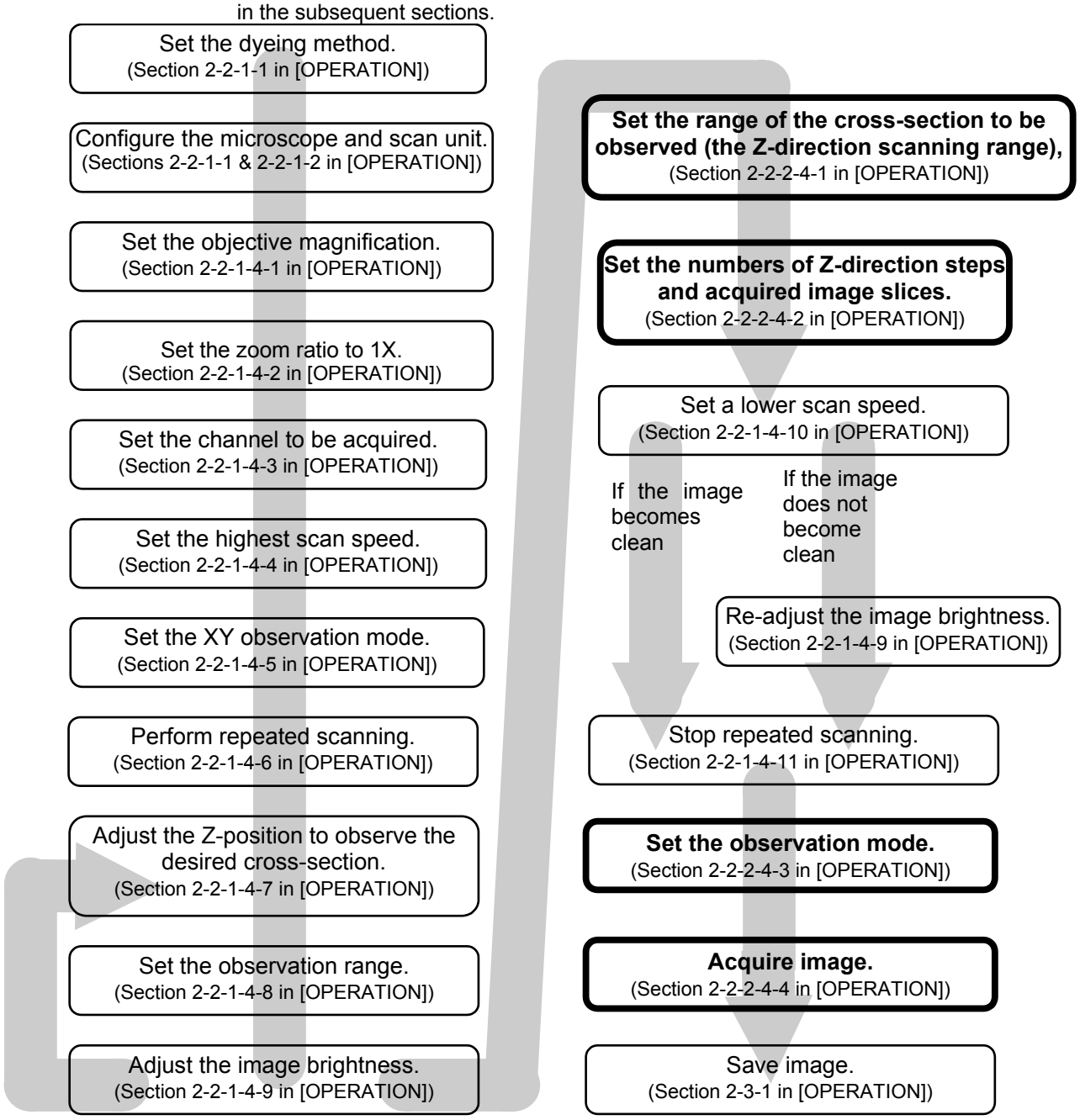
<Append Next> button
Acquires another image and appends it to the image acquired immediately before.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.
Click the <Series Done> button when it is not required to append an image.

2-2-2-4 XYZ Observation Mode

The description in this section will be focused on the image acquisition operations in the XYZ observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the cross-section to be observed (the Z-direction scanning range),
(Section 2-2-2-4-1 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-4-2 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-4-3 in [OPERATION])
Acquire image.
(Section 2-2-2-4-4 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

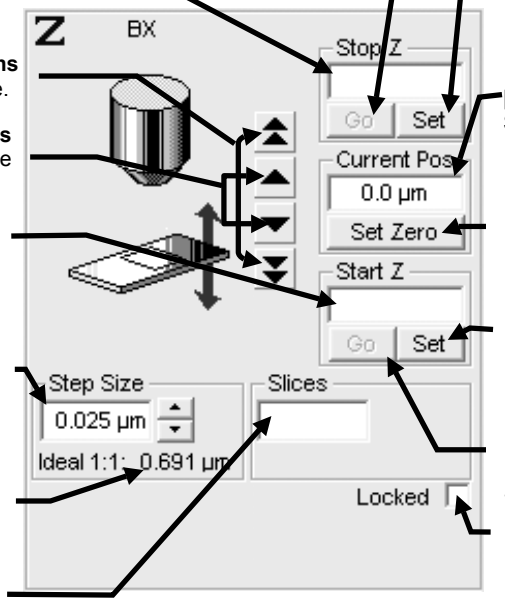
<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.



<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-16 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

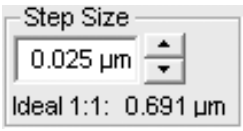
3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).

4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the numbers of steps and acquired image slices

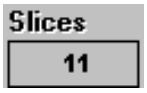


[Step Size] text box

1. Set the number of steps using the <▲> or <▼> button in the [Step Size] text box.

TIP

The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



[Slices] text box

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

3 Setting the observation mode

1. In the [Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Acquire] panel, select the XYZ observation mode option button.

4 Acquiring image

1. Click the <XYZ> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

While acquiring an image in the XYZ observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Resume> button
Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

5 Appending image

XYZ image can be added after the image acquisition.

Immediately after acquisition of an image in the XYZ observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

<Append Next> button

Acquires another image and appends it to the image acquired immediately before.

<Series Done> button

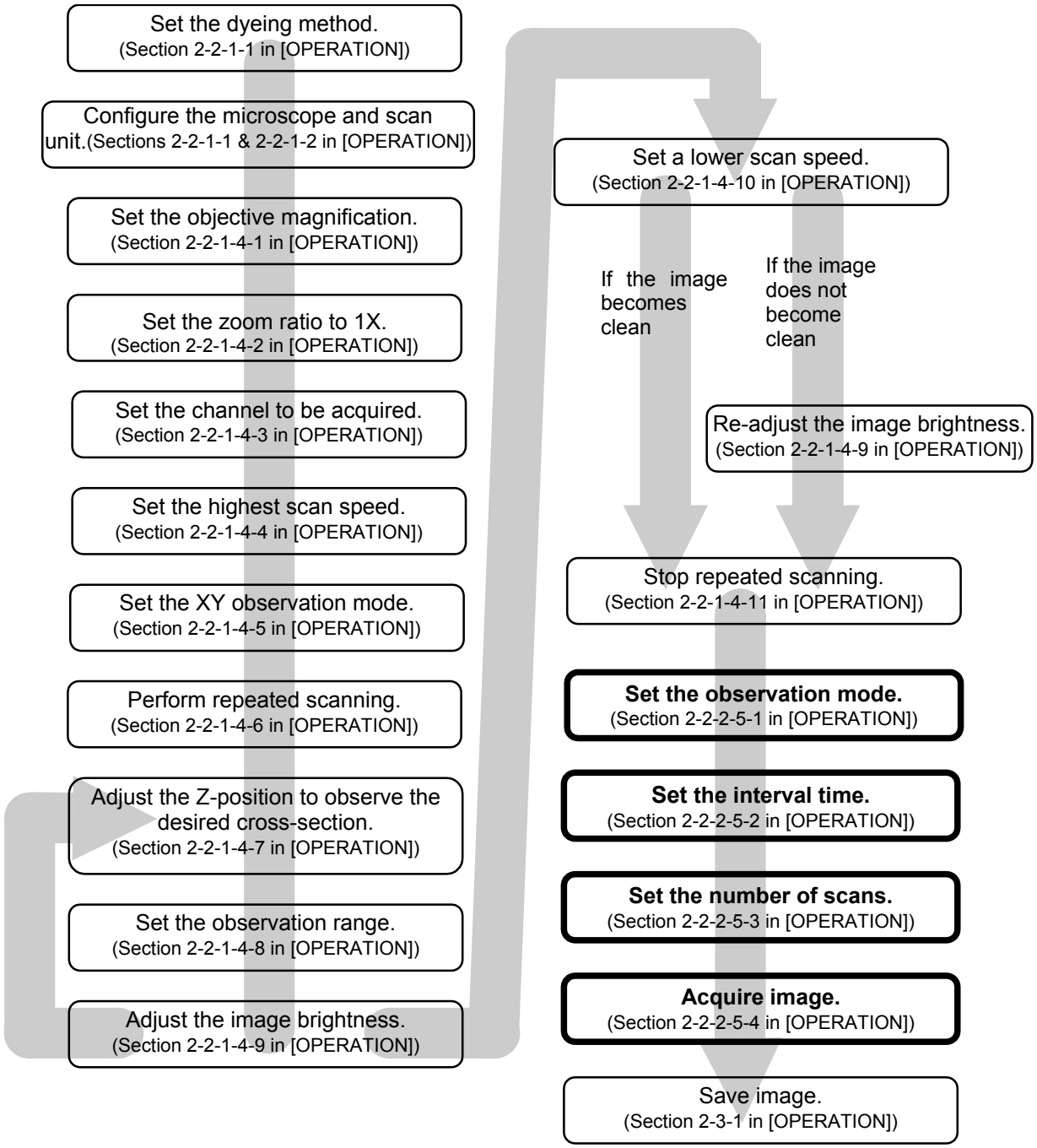
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.

Click the <Series Done> button when it is not required to append an image.

2-2-2-5 XYT Observation Mode

The description in this section will be focused on the image acquisition operations in the XYT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-5-1 in [OPERATION])
Set the interval time.
(Section 2-2-2-5-2 in [OPERATION])
Set the number of scans.
(Section 2-2-2-5-3 in [OPERATION])
Acquire image.
(Section 2-2-2-5-4 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

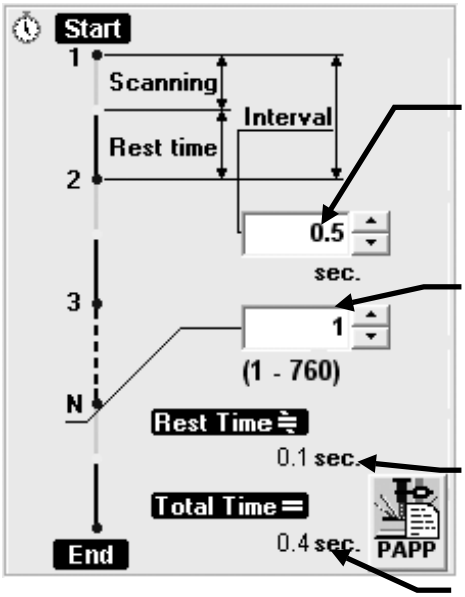
1 Setting the observation mode

1. In the [Mode] group box in the [Acquire] panel, select the [Surface] option button.

2. In the [Acquire] panel, select the XYT observation mode option button.

2 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. A panel as shown below will be displayed.



[Interval] text box

Set the interval time using the <▲> or <▼> button or by input from the keyboard.

[N] text box

Set the number of scans using the <▲> or <▼> button or by input from the keyboard.

Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

[Rest Time] text box

Shows the time after end of acquisition of an image until the start of next acquisition.

[Total Time] text box

Shows the total time required for image acquisitions.

Fig. 2-17 [Time Series] Sub-panel

2. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.

In this case, the [Interval] text box shows “Free Run” message.

Using <▼> button also sets the [Interval] text box to “Free Run”.

3 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

4 Acquiring image

Click the <XYT> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

When the built-in power supply for transmitted light illumination is used for a long period in the XYT mode, the metallic parts may be expanded by heat, causing the focusing to be deviated. To acquire precise image data, it is recommended to set the power switch of the transmitted light illumination to “O” (OFF).

While acquiring an image in the XYT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

5 Appending image

XYT image can be added after the image acquisition.

Immediately after acquisition of an image in the XYT observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

<Append Next> button

Acquires another image and appends it to the image acquired immediately before.

<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.

Click the <Series Done> button when it is not required to append an image.

2-2-2-6 XYZT Observation Mode

The description in this section will be focused on the image acquisition operations in the XYZT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.

Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])

Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])

Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])

Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])

Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])

Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])

Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])

Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])

Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])

Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])

Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])

Set the range of the cross-section to be observed (the Z-direction scanning range).
(Section 2-2-2-6-1 in [OPERATION])

Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-6-2 in [OPERATION])

Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])

If the image becomes clean

If the image does not become clean

Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])

Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])

Set the observation mode.
(Section 2-2-2-6-3 in [OPERATION])

Set the interval time.
(Section 2-2-2-6-4 in [OPERATION])

Set the number of scans.
(Section 2-2-2-6-5 in [OPERATION])

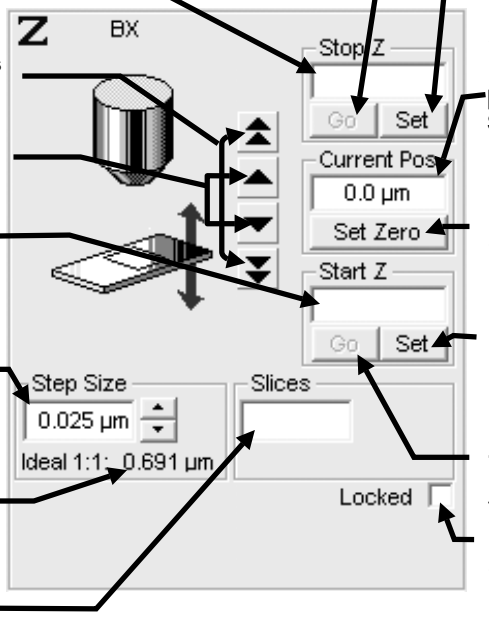
Acquire image.
(Section 2-2-2-6-6 in [OPERATION])

Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-18 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

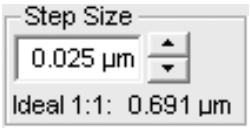
3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).

4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the numbers of steps and acquired image slices

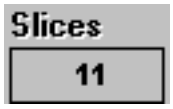


[Step Size] text box

1. Set the number of steps using the <▲> or <▼> button in the [Step Size] text box.

TIP

The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



[Slices] text box

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.

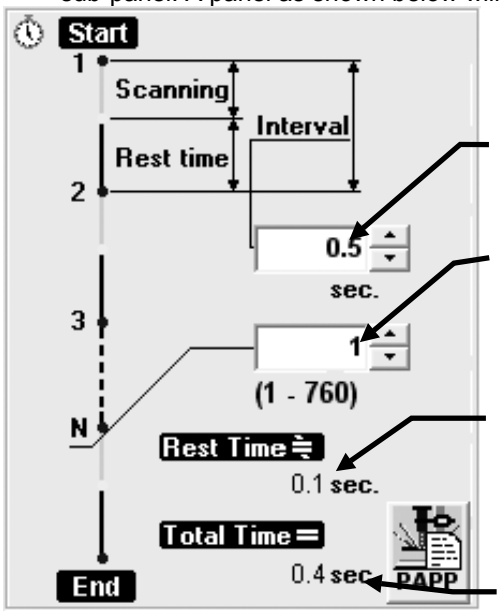
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

3 Setting the observation mode

1. In the [Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Acquire] panel, select the XYZT observation mode option button.

4 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. A panel as shown below will be displayed.



[Interval] text box
Set the interval time using the <▲> or <▼> button or by input from the keyboard.

[N] text box
Set the number of scans using the <▲> or <▼> button or by input from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

[Rest Time] text box
Shows the time after end of acquisition of an image until the start of next acquisition.

[Total Time] text box
Shows the total time required for image acquisitions.

Fig. 2-19 [Time Series] Sub-panel

2. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.
In this case, the [Interval] text box shows “Free Run” message.
Using <▼> button also sets the [Interval] text box to “Free Run”.

5 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

6 Acquiring image

1. Click the <XYZT> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.
   When the built-in power supply for transmitted light illumination is used for a long period in the XYZT mode, the metallic parts may be expanded by heat, causing the focusing to be deviated. To acquire precise image data, it is recommended to turn off the power of the transmitted light.

While acquiring an image in the XYZT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Resume> button
Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

7 Appending image

XYZT image can be added after the image acquisition.

Immediately after acquisition of an image in the XYZT observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

<Append Next> button
Acquires another image and appends it to the image acquired immediately before.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.
Click the <Series Done> button when it is not required to append an image.

2-2-3 Differences in Image Acquisition Method Between Fluorescent and Transmitted Images

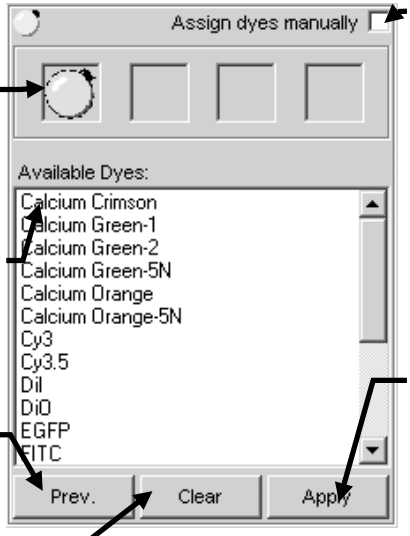
2-2-3-1 Monochrome Image

The wavelength obtained by monochrome dyeing can be acquired and observed as an image of a channel (either Ch1, Ch2, Ch3 or Ch4).

The subsequent description deals with the differences in operation between the monochrome dyeing and dual-fluorochrome dyeing.

- Set the dyeing method

  1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

[Available Dyes] list box

Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button

Clear the set dyeing method.

[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-20 [Dyes] Sub-panel

  2. Select the specimen dyeing method in the [Available Dyes] list box in the [Selected Dyes] group box, and drag the selected method into the field immediately above the list box.

  3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

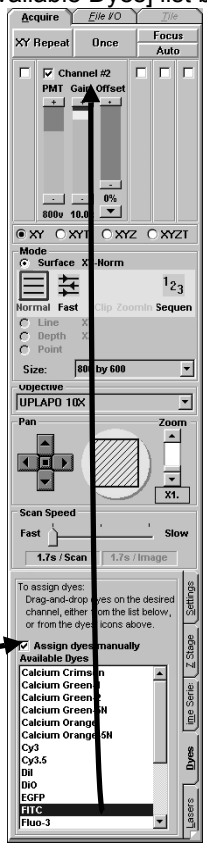
TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

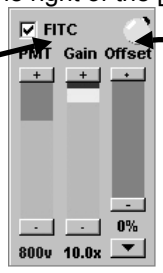
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Icon

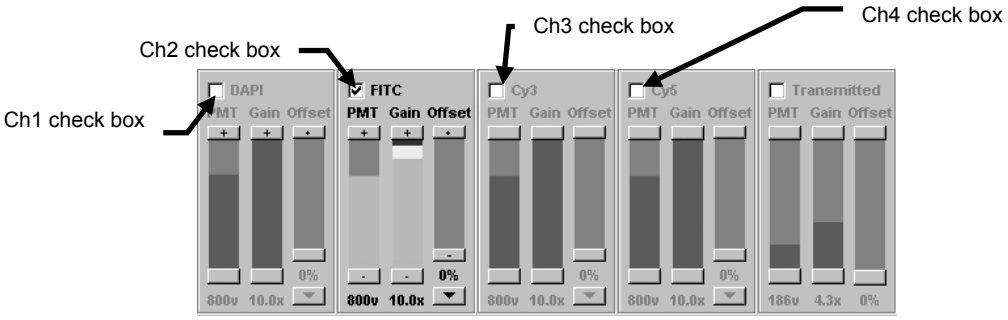
Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

- With types of dyeing of the specimen to observe, the barrier filter will be set automatically to the optical path.

  To change the barrier filter types, see section 1-3-2-3, “Configuring the Filters (When using a filter system)” and change by [Optical System Configuration] window.

- Make the channel ready for image acquisition.

  In the [Acquire] panel, make sure that the check box in the [Ch1]/[Ch2]/[Ch3]/[Ch4] group box that shows the dyeing method is check-marked to indicate that the corresponding channel is ready for image acquisition.



- Set the image brightness.

  Adjust the image brightness of the acquisition channel by using the [PMT], [Offset] and [Gain] LED sliders in the [Acquire] panel. For details, see to section 2-2-1-4-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

2-2-3-2 Dual-Fluorochrome Image

The wavelength obtained by dual-fluorochrome dyeing can be acquired and observed as images of the channels (Ch1 or Ch2).

The subsequent description deals with the differences in operation between the monochrome dyeing and dual-fluorochrome dyeing.

- Set the two or more dyeing methods.

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

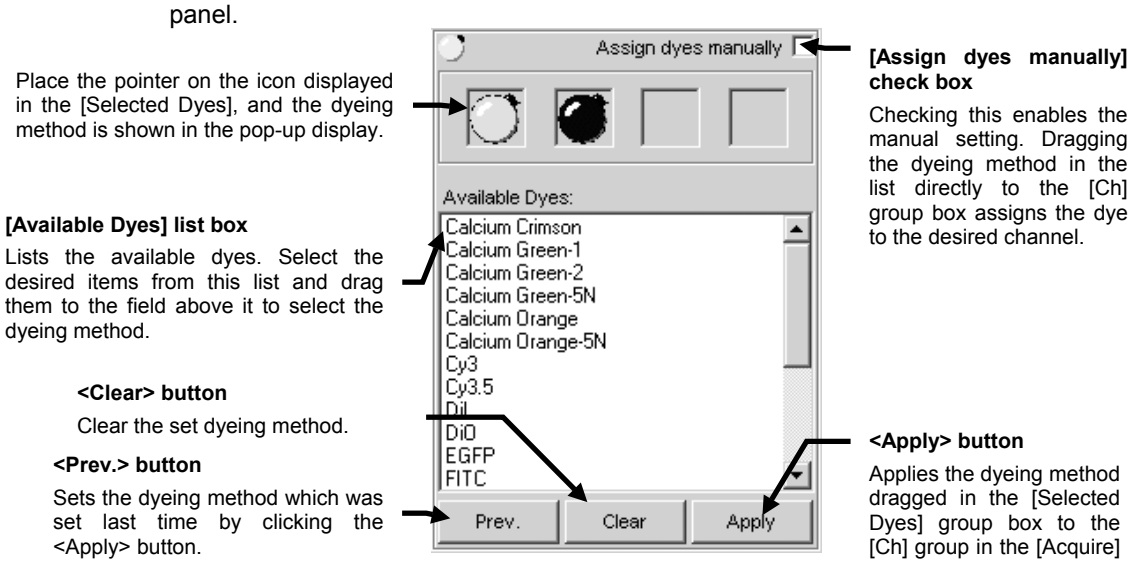

Assign dyes manually
Available Dyes:
Calcium Crimson
Calcium Green-1
Calcium Green-2
Calcium Green-5N
Calcium Orange
Calcium Orange-5N
Cy3
Cy3.5
DiI
DiO
EGFP
FITC
Prev.
Clear
Apply
Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.
[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.
[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.
<Clear> button
Clear the set dyeing method.
<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.
<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-21 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

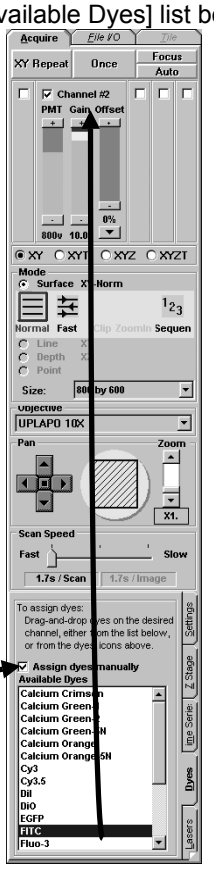
TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

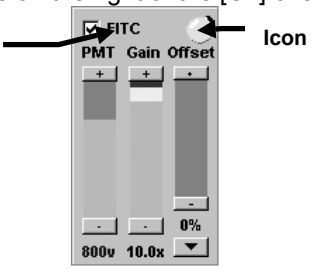
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set

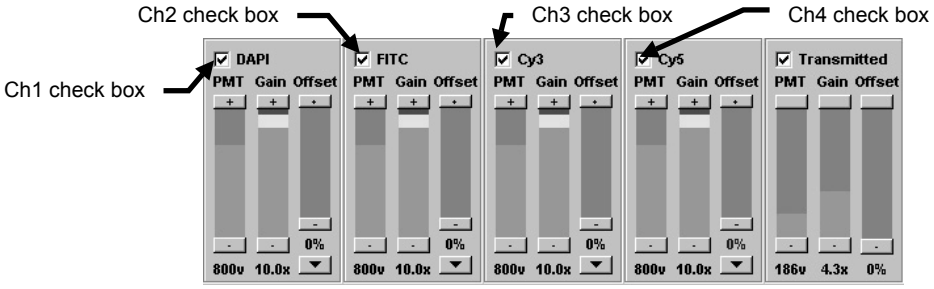


Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

- With types of dyeing of the specimen to observe, the Barrier Filters will be set automatically to the optical path.
  To change the Barrier Filter types, see to section 1-3-2-6, “Configuring the Filters” and change by [Optical System Configuration] window.

- Make the channels ready for image acquisition.
  In the [Acquire] panel, make sure that the check box in the [Ch1]/[Ch2]/[Ch3]/[Ch4] group boxes that show the dyeing methods are check-marked to indicate that the corresponding channels are ready for image acquisition.



- When observing a fluorescence image(s) simultaneously, also set the brightness of the fluorescence image(s).
  Adjust the brightness of the image of each channel by using the [PMT], [Offset] and [Gain] LED sliders in the [Acquire] panel. For details, see section 2-2-1-4-9, “Adjusting the Image Brightness”.

2-2-3-3 Transmitted Image

Images obtained by transmitted light observation can also be acquired or observed simultaneously with images obtained by fluorescence observation.

- When observing fluorescence images simultaneously, set the dyeing method.

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

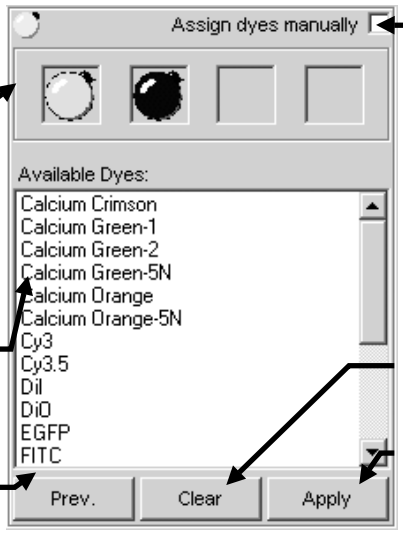
Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

[Available Dyes] list box

Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.



[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

<Clear> button

Clear the set dyeing method.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-22 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

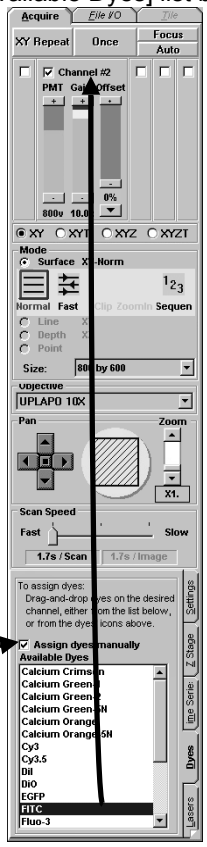
TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

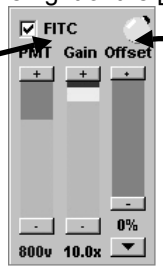
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

- With types of dyeing of the specimen to observe, the Barrier Filters will be set automatically to the optical path.
  To change the Barrier Filter types, see section 1-3-2-6,"Configuring the Filters " and change by [Optical system Configuration] window.

- Turn off the transmitted light bulb of the microscope.
  1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

<Tr. Lamp> button
Turns off the transmitted light bulb of the microscope.
(The lamp lights when the button is in the pressed status.)

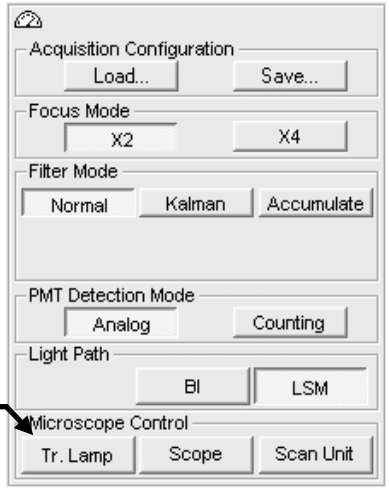
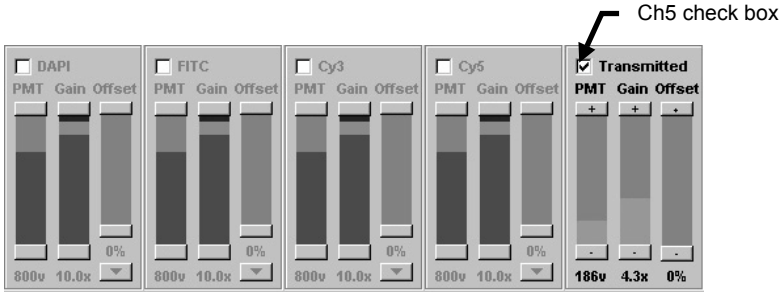


Fig. 2-23 [Settings] Sub-panel

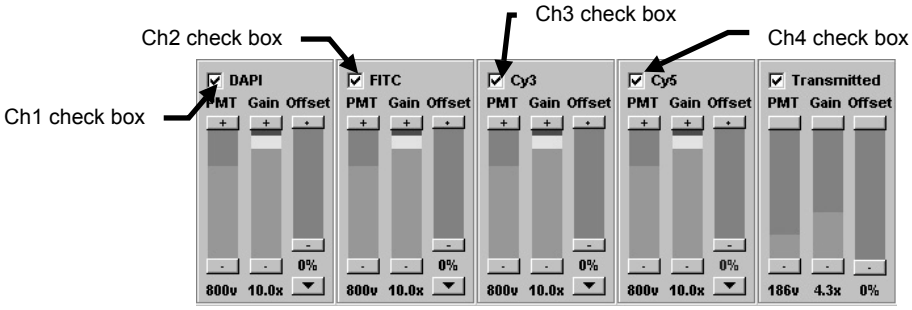
  2. Click the <Tr. Lamp> button to turn the lamp off (set the button to the non-pressed status).

- In the [Acquire] panel, make sure that the [Transmitted] check box in the [Ch5] group box is check-marked to indicate that Ch3 is ready for image acquisition.



- When observing a fluorescence image simultaneously, set the required channel ready for acquisition of fluorescence image.

  Make sure that the check box showing the dyeing method in the [Ch1]/ [Ch2]/[Ch3]/[Ch4] group box is check-marked to indicate that the corresponding channel is ready for image acquisition.



- Set the brightness of the transmitted image.

  Please use PMT, Offset and Gain LED sliders in [Ch5] group box to adjust the brightness of the transmitted image. For details, see section 2-2-1-4-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

- When observing a fluorescence image(s) simultaneously, also set the brightness of the fluorescence image(s).

  Adjust the brightness of the image of the transmitted image acquisition channel(s) by using the [PMT], [Offset] and [Gain] LED sliders in its group box in the [Acquire] panel. For details, see section 2-2-1-4-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

2-2-4 Image Acquisition by Rotating It (Rotation Scan)

Rotation scan enables image acquisition with tilting the field of viewed.

NOTE **Rotation Scan can not be used with Free Line Scan.**

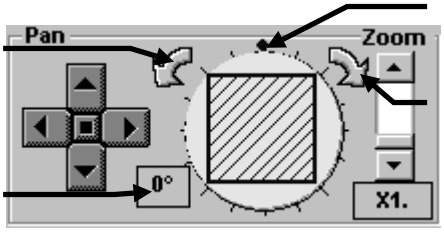
1. Acquire an image in the XY observation mode. For the operating procedure, see section 2-2-1, “Image Acquisition in XY Observation Mode”.

2. Set the image acquisition mode.
   For details, see sections 2-2-1, “Image Acquisition in XY Observation Mode”, 2-2-2, “Image Acquisition in Other Observation Modes”, 2-2-5, “Image Acquisition of Only the Rectangular Position (Clip Scan)”, 2-2-6, “Image Acquisition by Magnifying the Rectangular Position (Zoom-In Scan)”, 2-2-7, “High-Speed Image Acquisition” and 2-2-8, “Acquiring Finer Image (Sequential Scan)”.

3. Specify the image rotation angle using the rotation arrow buttons on both sides of the circuit in the [Pan] group box in the [Acquire] panel.

Assuming that the angle at the top of the circle is 0°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Displays the image rotation angle. The angle can also be specified by direct entry.



Dragging the blue handle along the circuit allows the acquisition area to be tilted.

Assuming that the angle at the top of the circle is 0°, tilts the acquisition area by 1° per step in the counterclockwise direction.

4. Acquire the image by clicking the <XYZ>, <XYT> or <XYZT> button.

2-2-5 Image Acquisition of Only the Rectangular Position (Clip Scan)

The Clip Scan mode limits the image acquisition range to the range to be observed and acquires the image of only that range.

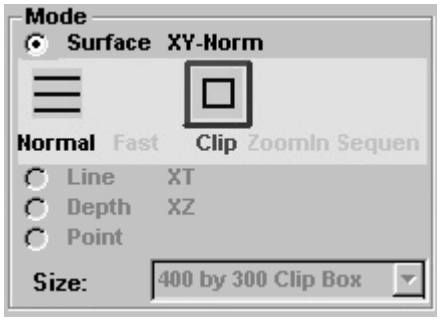
Applying this mode in an image acquisition mode involving large amount of data, such as the XYZ, XYT and XYZT observation modes, makes it possible to acquire data of a long period of time in a small file size.

For example, let us assume that an 800 x 600 pixel image is displayed in the [Live] panel and the image acquisition area is limited to an area with 400 x 300 pixels (halved in both X and Y directions).

When the image is acquired in this mode, the size per pixel on the specimen is identical to that before start of this mode.

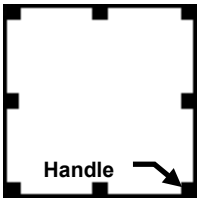
1. Acquire an image in the XY observation mode. For the detailed operation method, see section 2-2-1, “Image Acquisition in XY Observation Mode”.

2. Select the [Surface XY-Norm] option button in the [Mode] group box in the [Acquire] panel, then select <Clip> from the list displayed below it.
   A frame indicating the scanning range will be displayed in the [Live] panel.
   In the initial setting, the image is displayed with both the X and Y sizes set to half the original sizes.



3. Move the frame around the range to be observed. The frame can be moved by placing the mouse pointer inside it and dragging.

4. Change the frame size. To change the frame size, click a point inside the frame with the mouse pointer. When square handles are displayed on the frame edges, place the mouse pointer on one of them and drag it.



5. Change the inclination angle of the frame. Click the mouse right button inside the frame to display the pop-up menu, and select [Rotate] in it.

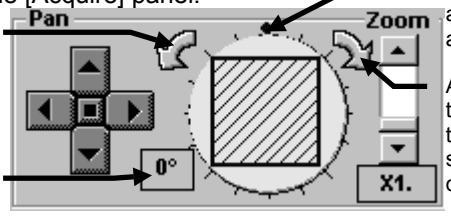
6. The inclination angle of the frame can be varied according to the mouse pointer movement.

   Place the mouse pointer on an inclining position and click the mouse left button to fix the frame inclination angle.

7. When using hardware that is compatible with rotation scan (FV5-IO3), specify the image rotation angle using the rotation arrow buttons on both sides of the circuit in the [Pan] group box in the [Acquire] panel.

Assuming that the angle at the top of the circle is 0°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Displays the image rotation angle. The angle can also be specified by direct entry.



Dragging the blue handle along the circuit allows the acquisition area to be tilted.

Assuming that the angle at the top of the circle is 0°, tilts the acquisition area by 1° per step in the counterclockwise direction.

8. Acquire images by clicking the <XYZ>, <XYT> or <XYZT> button.

9. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Mode] group box to set the scanning range to the original setting.

NOTE

It is not permitted to change the scanning range after having acquired images. If you want to change the scanning range again, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Mode] group box to set the scanning ragne to the original setting, acquire an image in the XY observation mode, then select <Clip> in the [Mode] group box and reselect the scanning range.

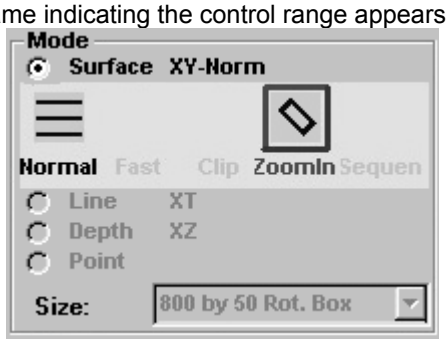
2-2-6 Image Acquisition by Magnifying the Rectangular Position (Zoom-In Scan)

The Zoom-In Scan mode limits the scanning area to the range to be observed and acquires the image of that area by magnifying it.

When the image is acquired in this mode, the number of pixels in the X direction becomes identical to the image size before start of this mode, and the magnification applied is determined according to the size of the limited range. High-speed image acquisition can be made possible by decreasing the number of pixels in the Y direction.

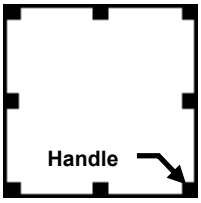
1. Acquire an image in the XY observation mode. See section 2-2-1, “Image Acquisition with XY observation for operation procedure.

2. Select <ZoomIn> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel. A frame indicating the control range appears in the [Live] panel.



3. Move the frame to the area to be observed. To move the frame, place the mouse pointer inside it and drag the mouse.

4. Change the frame size. Place the mouse pointer inside the frame and click to display square handles around the frame. Then place the mouse pointer on one of these handles and drag it to change the frame size. (Here the X:Y ratio of the frame is not changed.)



5. Change the number of pixels in the Y direction. Right click the mouse on the frame to display a pop-up menu and select [Size] in it to display square handles around the frame. Place the mouse pointer on one of these handles and drag it to change the number of pixels. Note that the X:Y ratio of the frame is not changed during this.
   If high-speed image acquisition is required, decrease the number of pixels in the Y direction.

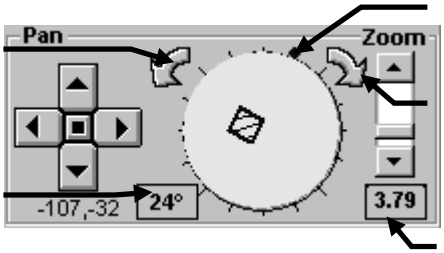
6. Change the inclination angle of the frame. Click the mouse right button inside the frame to display the pop-up menu, and select [Rotate] in it.

7. The inclination angle of the frame can be varied according to the mouse pointer movement.
   Place the mouse pointer on an inclining position and click the mouse left button to fix the frame inclination angle.

8. When using hardware that is compatible with rotation scan (FV5-IO3), specify the image rotation angle using the rotation arrow buttons on both sides of the circuit in the [Pan] group box in the [Acquire] panel.

Assuming that the angle at the top of the circle is 0°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Displays the image rotation angle. The angle can also be specified by direct entry.



Dragging the blue handle along the circuit allows the acquisition area to be tilted.

Assuming that the angle at the top of the circle is 0°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Actual zoom value appears.

9. Acquire the image with the buttons such as the <XYZ>, <XYT>, <XYZT>buttons.

10. After acquiring the image, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-7 High-Speed Image Acquisition

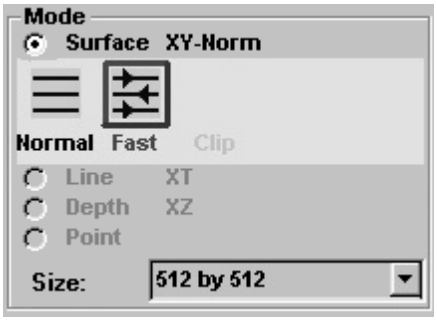
An image can be acquired in 0.25 second.

This image acquisition mode is valid under the following condition:

- Max. 2 channels
- Image size: 512 x 512 pixels
- Zoom ratio of 2X or more, with setting in 2X steps

1. Check one or both of the [Ch] check boxes in the [Ch] group box in the [Acquire] panel.

2. Select the [Surface XY-None] option button in the [Mode] group box in the [Acquire] panel. Then select <Fast> in the list shown below.



[512 by 512] is set automatically in the [Size] drop-down list in the [Mode] group box. "X2" is set and shown in gray-out display automatically in the [Zoom] scale in the [Pan] group box.

3. To change the zoom ratio, use the [Zoom] scale in the [Pan] group box.

<XY Repeat> button

4. Select any button to acquire image such as the <XY Repeat> button.

TIP High-speed image acquisition is available in the XY, XYT, XT observations.

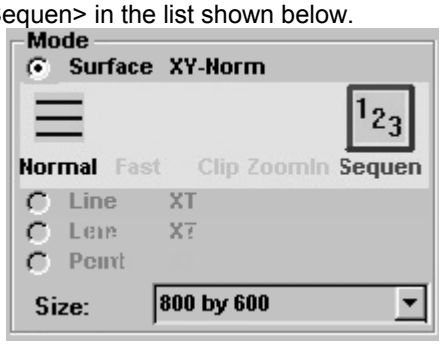
5. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Mode] group box to set the scanning range to the original setting.

2-2-8 Image acquisition to prevent crosstalk between fluorescence (Sequential Scan)

An image which is suppressed crosstalk can be acquired sequentially by combination of excitation laser and those image acquisition channel.

With this image capturing method, the image of a multiple-dyed specimen can be obtained by sequentially acquiring image slice of each type of fluorescence.

1. Select the [Surface XY-Note] option button in the [Mode] group box in the [Acquire] panel, then select <Sequen> in the list shown below.



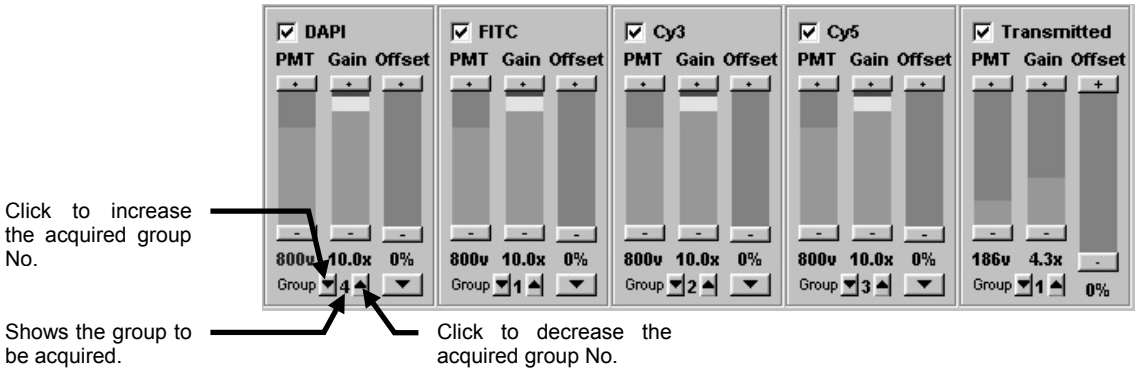
Selecting <Sq Clp>, <Sq Rct>, or <Line Sq> enables the image acquisition of the desired rectangle area or that of the rectangle area at desired angle, or a line sequential scan..

Line Sequential Scan

Ordinary sequential scan is performed for every one frame for every group, whereas line sequential scan is performed for every one line for every group. Therefore, line sequential scan is of advantage in minimizing the time lag between wavelength and acquiring a fewer cross-talk image.

2. Select the observation mode with the option buttons in the [Acquire] panel.

3. [Group] (which means each laser) and the <▲> or <▼> buttons appear on the lower part of each [CH] group box.



4. Set the group number according to the reagent in use with the <▲> or <▼> buttons. This setting is not required if the desired number is already displayed.

TIP Image acquisition starts with the smallest group number set.

NOTE **To prevent unwanted color fading, it is recommended to acquire the image from the reagents with longer wavelengths.**

5. Click the <Seq. Once> button to acquire the image.

6. When transmitted observation is also attempted, the [FLUOVIEW] dialog box and the [Lasers] sub-panel appear to specify the laser type which has not been specified yet.

Fig. 2-24 [FLUOVIEW] Dialog Box

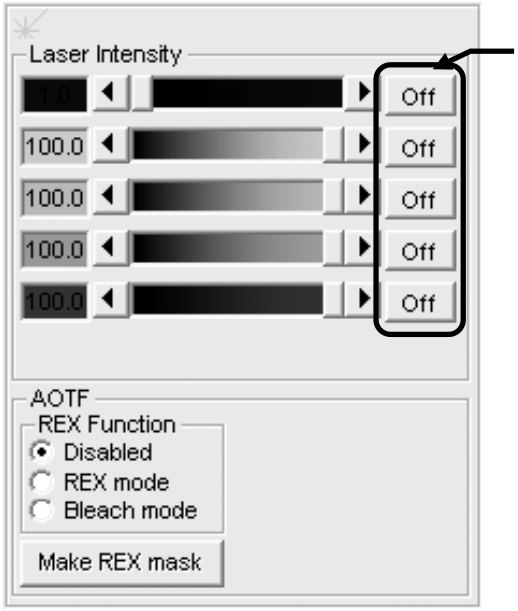


Fig. 2-25 [Lasers] Sub-panel

7. Among the laser <Off> buttons on the right of the [Laser Intensity] scales in the [Lasers] group box, click the <Off> button of the laser you want to use in transmitted observation. This should change the <Off> button to the <Rdy> button (displayed in the pressed-in condition).

8. Click the <OK> button in the [FLUOVIEW] dialog box.
   Image acquisition starts.

9. Adjust the brightness with the [PMT], [Offset] and [Gain] LED sliders in the [Ch] group box in the [Acquire] panel. See section 2-2-1-4-9, “Adjusting the Image Brightness” for details.

10. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Mode] group box to set the scanning range to the original setting.

2-2-8-1 Virtual Channel Function

The virtual channels refer to the simulated detection channels produced when a single detector switched in sequential scan. The virtual channels make it possible to perform sequential scan by switching the lasers and filters according to the preset condition by increasing the number of detection channels.

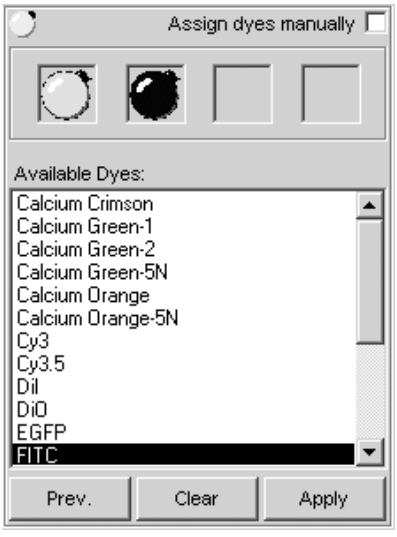
NOTE

Up to 6 virtual channels can be set up.
If 6 channels are already used by means of option units, it is no longer possible to set up a virtual channel.

Operation Example

The following procedure deals with the case of observing FITC in CH1, PI in CH2 and Cy5 in a virtual channel.

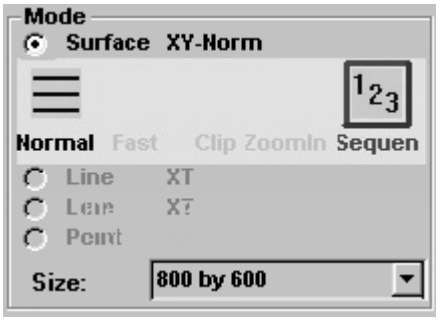
1. In the [Dyes] sub-panel of the [Acquire] panel, select the dyeing methods and click the <Apply> button. (Example: FITC and PI are selected.)
   For details, see step 2 in the procedure in section 2-2-1-1, “Configuring the Microscope”.



<Once> button

2. Click the <Once> button to acquire an image.

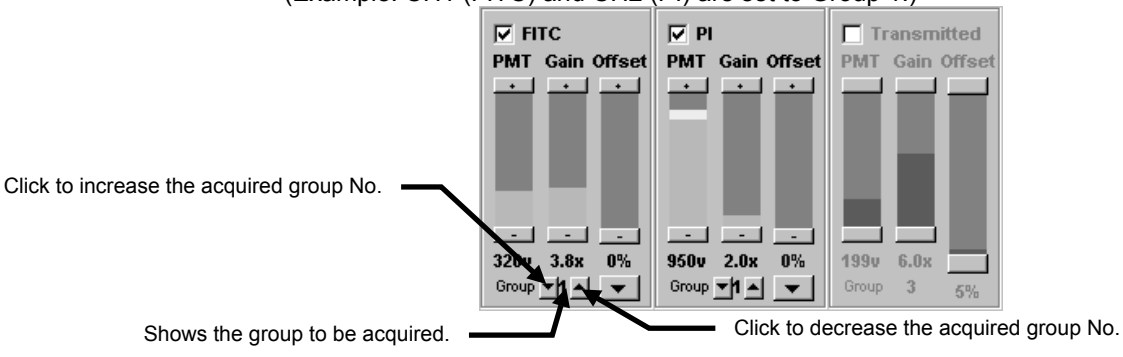
3. In the [Mode] group box in the [Acquire] panel, click the [Surface XY-Norm] option button, select <Sequen> from the list below it and select the scan mode to be used from the displayed icons.



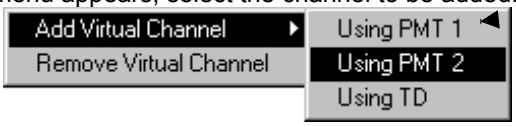
NOTE **Do not select <Line Aq> in the [Mode] group box in the [Acquire] panel because the virtual channels are not compatible with Line Sequential Scan.**

TIP If the observation mode is set to Sequential Scan, the [Dyes] sub-panel in the [Acquire] panel cannot be used.

4. Using the <▲> and <▼> buttons in [Group], set the group according to the reagent in use. This operation is not required if the current setting needs not be changed. (Example: CH1 (FITC) and CH2 (PI) are set to Group 1.)

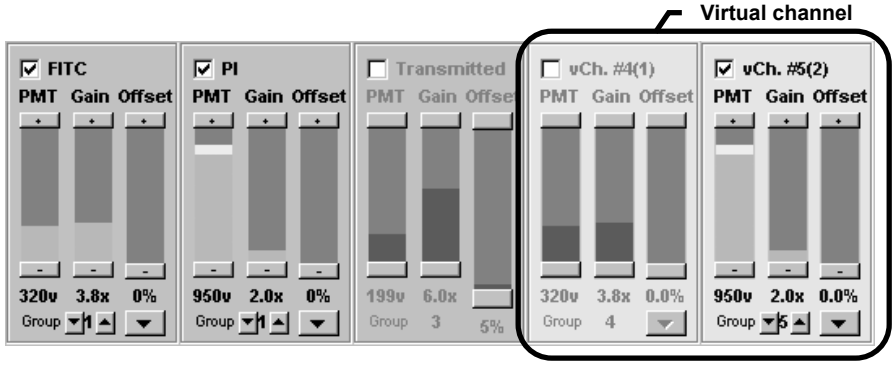


5. Right-click the mouse on the [Ch] group box on the upper part of the [Acquire] panel. When a pop-up menu appears, select the channel to be added.



Select the channel to which you want to add a virtual channel.

A virtual channel will be added in the [Ch] group box. (Example: When [Using PMT 1] and [Using PMT 2] are selected from the pop-up menus.)



NOTE

It is not permitted to set the physically identical channels (an ordinary channel and virtual channel) in the same group. (For example, ordinary channel 1 and virtual channel 1 cannot be set in the same group.)

TIP

Sequential Group means a set of image channel which is acquired in a sequential image acquisition.

Channel 1 and Virtual channel 1 needs to assign in deferent Sequential Group since those channel can not acquire at the same time.

6. Select <SU Control> button of the [Settings] sub-panel in the [Acquire] panel.

   In the [Optical System Configuration] window, select the conditions such as laser and filter settings of each group.

   For details on the [Optical System Configuration] window, see section 1-3-2-6, “Setting the Filters”.

   (Example: Set the group No. to [2] in the [Group #] drop-down list of [For Seq. Scan]. Then open the [Group] drop-down list of vCh (2) in the [Virtual Channel] group box and select [2].

   In the [Dye] drop-down list of vCH (2), select [CY5].

   In the [Excitation DM] drop-down list, select [DM488/543/633].

   In the [Beam Splitter] drop-down list, select [Glass].

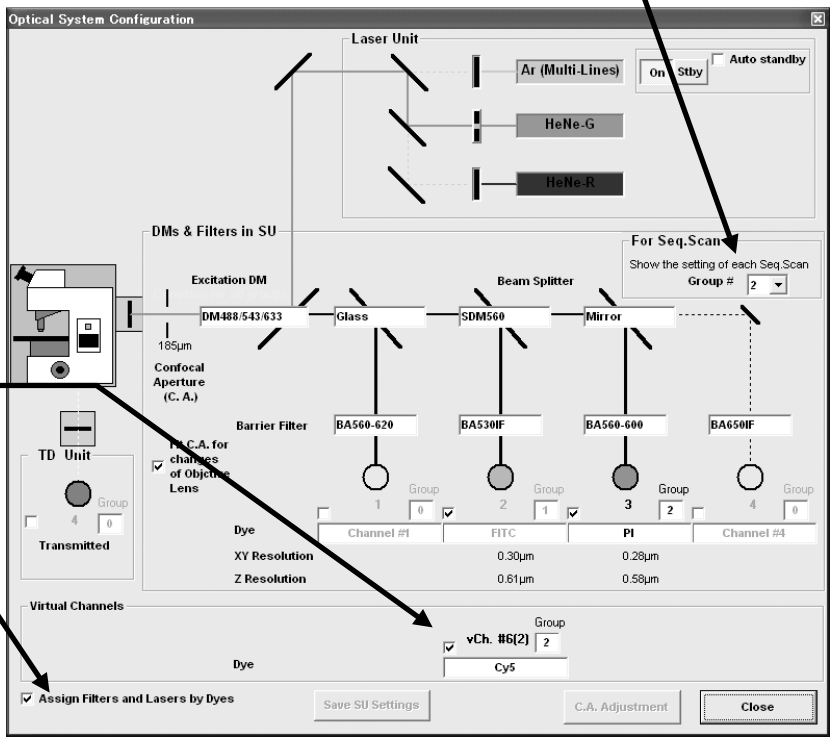
   In the [Barrier Filter] drop-down list for CH2, select [BA660IF].

   In the [Laser Unit] group box, click the picture of shutter and engage the HeNe-R laser in the light path.

   After completing the setup, select the <Close> button to close window.

[For Seq. Scan] group box

Use this group box when performing sequential scan. Select the group to be set in the [Group] drop-down list and set the laser and filter settings in the [DMs & Filters in SU] group box to change the settings of each group.



[Virtual Channels] group box

Show the virtual channels.

Click a channel checkbox to check it makes the virtual channel available. Select the dyeing method from the drop-down list and select the group No. in the [Group] drop-down list.

[Assign Filters and Laser by Dye] check box

When checked, settings of filter and laser will be automatically done according to selection of dye method.

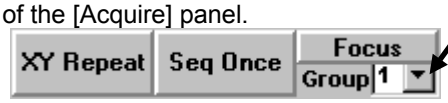
NOTE **Setting will disappear if <Normal> is selected at [Mode] group box of [Acquire] panel after setting virtual channel. It is useful that the setting is saved with use of <Save> button at [Acquisition Settings] group box on [Settings] sub panel of [Acquire] panel before selecting <Normal>.**

NOTE **It is not permitted to set the excitation dichroic mirror differently for each channel. The excitation dichroic mirror set up at the last is applied to all groups.**

TIP When the laser to be used with a group has not been set, the [Laser Unit] dialog box shows “No Laser has assigned yet”.
In this case, set the laser in the [Laser Unit] group box.

TIP If Sequential Scan is set already, the <C.A. Adjustment> button in the [Optical System Configuration] window cannot be selected.

7. Adjust the image brightness for every group.
   Select the group to adjust the image brightness in the [Group] drop-down list displayed on the upper part of the [Acquire] panel.



[Group] drop-down list
Select the sequential scan group.

TIP Select a group before repeated scanning. The group selection cannot be changed during repeated scanning.

<XY Repeat> button

<Focus> button

8. Click the <XY Repeat> or <Focus> button to perform repeated scanning in order to adjust the brightness of the channels.

Select a group before repeated scanning. The group selection cannot be changed during repeated scanning.

9. Adjust the brightness using the [PMT], [Offset] and [Gain] LED sliders in the [Ch] group box in the [Acquire] panel. For details, see section 2-2-1-4-9, “Adjusting the image brightness”.

10. Click the <STOP SCAN> button to stop repeated scanning.

11. Repeat steps 7 and 10 for each of the groups to be configured.

<Seq Once> button

12. Click the <Seq. Once> button to start sequential scan.

13. After image acquisition, click <Normal> below the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode. This hides the virtual channels from the display.

Only the image(s) of the current Sequential Group is updated when image is acquired by pressing <XY Repeat> button or <Focus>. The other Sequential Group image stays in [Live] panel.

2-2-9 Image Acquisition of a Line at Desired Angle

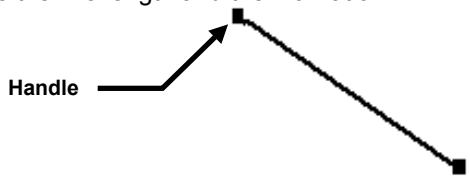
When the sample is not standing upright, the image of a line can be acquired by changing the angle.

1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, “Image Acquisition with XY Observation”.

2. Select any one of <Normal>, <Clip> or <ZoomIn> of [Line XT] option button or [Depth XZ] option button in [Mode] group box of [Acquire] panel.
   A line indicating the control range appears in the [Live] panel.

3. Move the line to the area to be observed. To move the line, place the mouse pointer on it and drag the mouse.

4. Change the line length and the inclination. Place the mouse pointer on the line and click to display square handles on the two extremities of the line. Then place the mouse pointer on either handle and drag it to change the line length and the inclination.



5. Acquire the image with the buttons such as the <XYZ>, <XYT>, <XYZT> buttons.

6. After acquiring the image, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

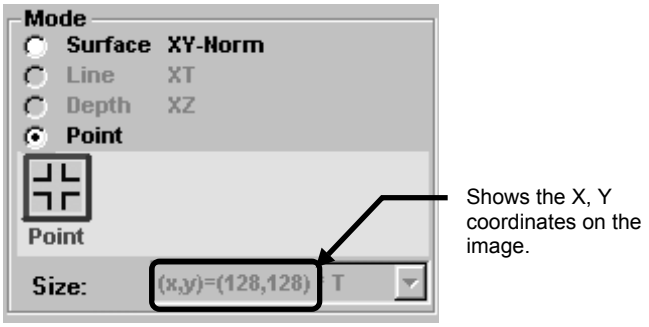
2-2-10 Display the change of image intensity (Point Scan)

The change of fluorescence intensity according to time-lapse can be displayed graphically by irradiating the laser to certain point on the image.

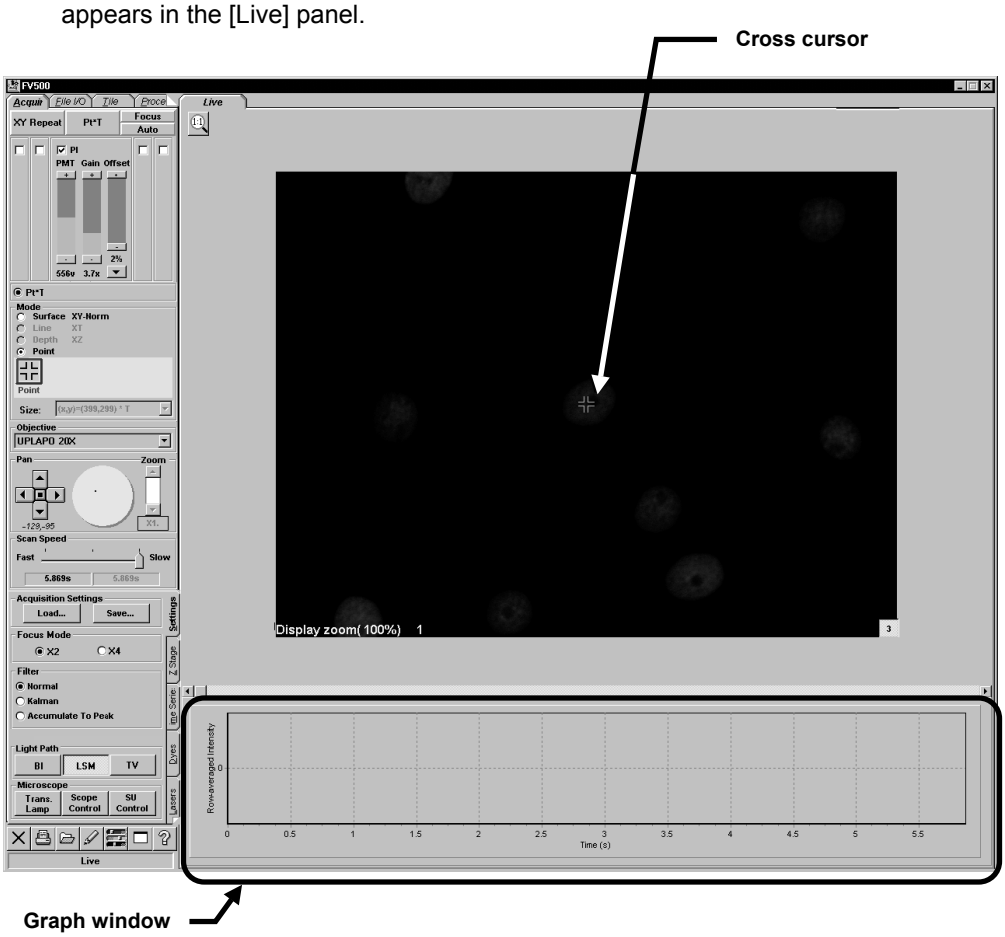
If you have FLUOVIEW TIME COURSE software, image acquisition can be started with buttons, keys or input of external trigger signals.

1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, “Image Acquisition with XY observation”.

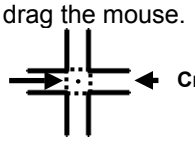
2. Select <Point> under the [Point] option button in the [Mode] group box in the [Acquire] panel.



The graph window showing the cross cursor for scanning and the intensity values appears in the [Live] panel.



3. Move the cross cursor to the area you want to observe. To move the cross cursor, place the mouse pointer on it and drag the mouse.

Scan the center of the cross cursor  **Cross cursor**

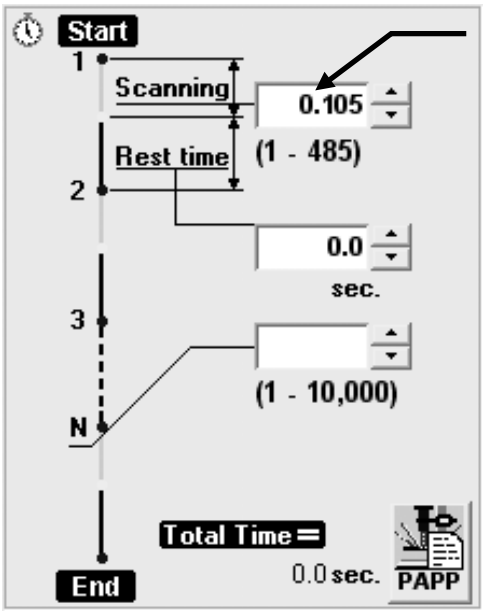
4. Set the scan speed for image acquisition.

   Set the speed in the [Scan speed] group box of the [Acquire] panel.

TIP

The speed recommended for point scan area as follows.
2μs(The scroll bar indicates “Fast” position. It is useful for the specimen changes rapidly.), 10μs, 100μs(The scroll bar indicates “Slow” position. It is useful for the specimen changes slowly.)

5. Set the time for measurement.
   From the page tabs on the bottom right of the [Acuire] panel, select the [Time Series] sub-panel.
   Set the time for measurement with the <▲> or <▼> button in the [Scanning] text box.



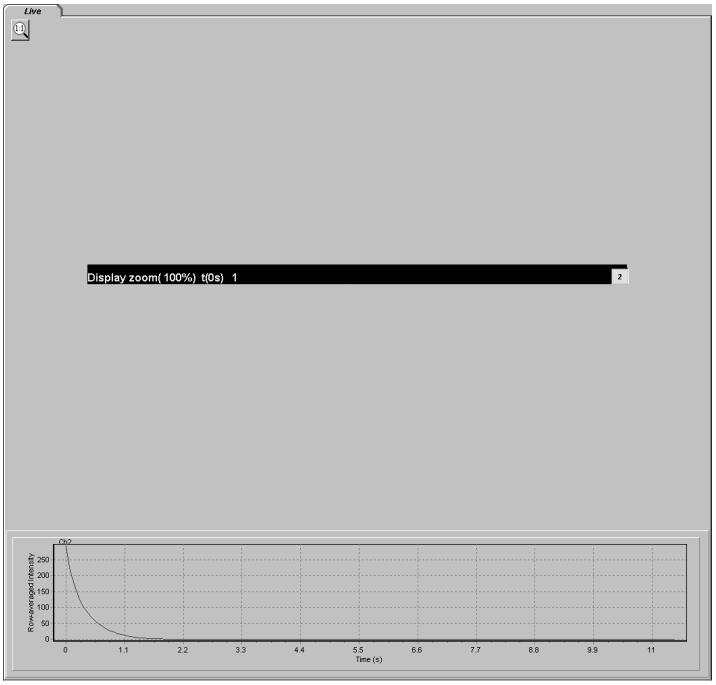
6. Set the starting method for image acquisition.
   (If you have FLUOVIEW TIME COURSE software)
   For setting procedure, see “FLUOVIEW TIME COURSE software user’s manual”.

7. Click the <Pt^{x}T> button to acquire the image.
   The image at the coordinates set in the [Live] panel appears in the image display area.

8. When image acquisition is completed, average values of intensity of each lines on the image acquired would be plotted on graph window.

TIP

The image shown in the [Live] panel after image acquisition is the image that acquired at the coordinates specified in the [Live] panel and arranged in the X direction, from the top left to the bottom right.
The image shown after image acquisition is the same a width as that in the X direction specified in the [Live] panel.



TIP

When a certain area is specified by dragging the mouse from the top left to the bottom right on the graph while pressing down its left button, the specified area can be magnified.

TIP

When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP

The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse on the magnified graph from the bottom left to the top right, the top right to the bottom left or the bottom right to the top left.

TIP

When click the mouse on the X or Y Axis on the graph window, the [Editing] dialog box appears and the graph parameters or the graph display method can be modified.

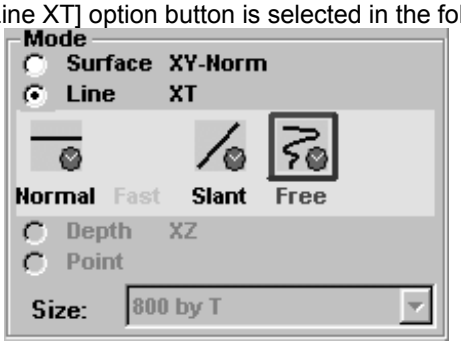
9. After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-11 Image Acquisition on Desired Line (XZ, XT or XZT Observation)

When the sample is in curved form, the image can be acquired by drawing a desired line on it.

1. Acquire an image in the XY observation mode. For detailed operation, see section 2-2-1, “Image Acquisition with XY observation”.

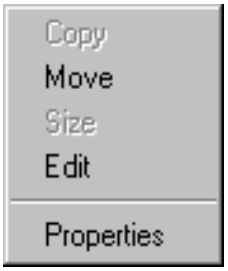
2. Select [Line XT] option button in the [Mode] group box in the [Acquire] panel or <Free> under the [Depth XZ] option button. A line indicating the scanning range appears in the [Live] panel.
   (<Free> under the [Line XT] option button is selected in the following Fig.)



TIP A line can be specified for one display.

3. Move the curved line to the area you want to observe. To move the curve, place the mouse pointer in the box and drag the mouse.

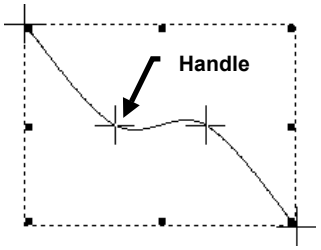
4. Change the curved line shape. Place the mouse pointer within the box and right click to display the pop-up menu. Then select <Edit>.



The cross shaped handles appear around the curve. Place the mouse pointer on either handle and drag it to change the line shape. The color of the mouse pointer is changed on the handle.

The handle can be added by clicking the mouse on desired coordinates.

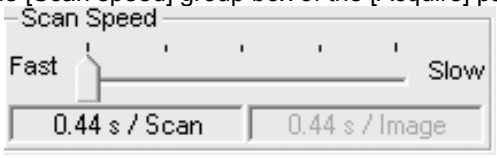
To delete the handle, right click the mouse on it.



Right click the mouse on the coordinates but the handles to fix the shape after the change.

5. Set the scan speed for image acquisition.

Set the speed in the [Scan speed] group box of the [Acquire] panel.



TIP

The speed recommended for this scan mode are as follows.

2μs: The scroll bar indicates “Fast” position. It is useful for the specimen changes rapidly.

4μs, 8μs : The scroll bar indicates “Slow” position. It is useful for the specimen changes slowly.

NOTE **When <Free> is selected at [Mode] group box on [Acquire] panel, the sampling speed cannot be changed.**

6. Set the interval time or the number of scans.
   The operating procedure is same as that in the XY observation mode. See section 2-2-2, “XT Observation Mode” for details.

TIP Up to 8000 times, images can be acquired. Set the number of scans in the [N] text box in the [Time Series] sub-panel.

7. Click <XZ>, <XT>, or <XZT> etc. to acquire the image.

TIP The image shown in the [Live] panel after image acquisition is the image that acquired at the curved line specified on the image and arranged uprightly in the X direction, from the top to bottom.
The image shown after image acquisition is the same a width as that in the X direction specified in the [Live] panel. (Same as that in the XT observation mode).

8. After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-12 Image Acquisition in the Laser Excitation Mode

When you use the FV system with AOTF (FV5-COMBA), the function described in this section is available.

In the FV system with AOTF, it is possible to cut excitation of laser except the region where scanning is performed. Moreover, it is also possible to set up a region of laser excitation to excite laser only to the target part.

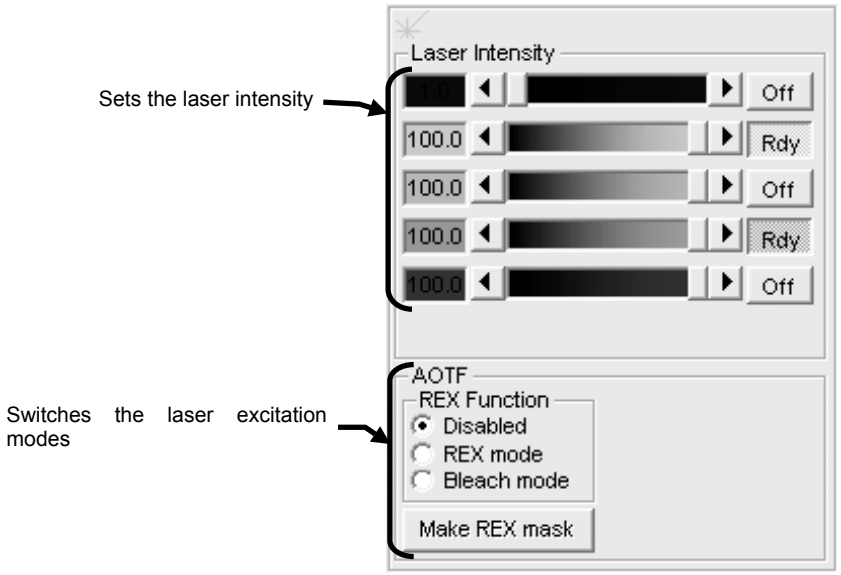
TIP REX represents the “region of laser excitation” in this section.

Three kinds of the laser excitation mode, Disable, REX, and Bleach, are selectable according to your purpose. And each mode can be switched in the XYT observation. In use of the REX or Bleach mode, a setup of REX mask file is required.

Disable mode: An ordinary mode to acquire an image of specified size.

REX mode: The mode which sets the REX mask file to excite laser only to the target part and acquire image. The same laser setup as Disable mode is applied.

Bleach mode: The REX mask file other than that of the REX mode can be set. And each laser setup can also be obtained apart from the Disable/REX mode.

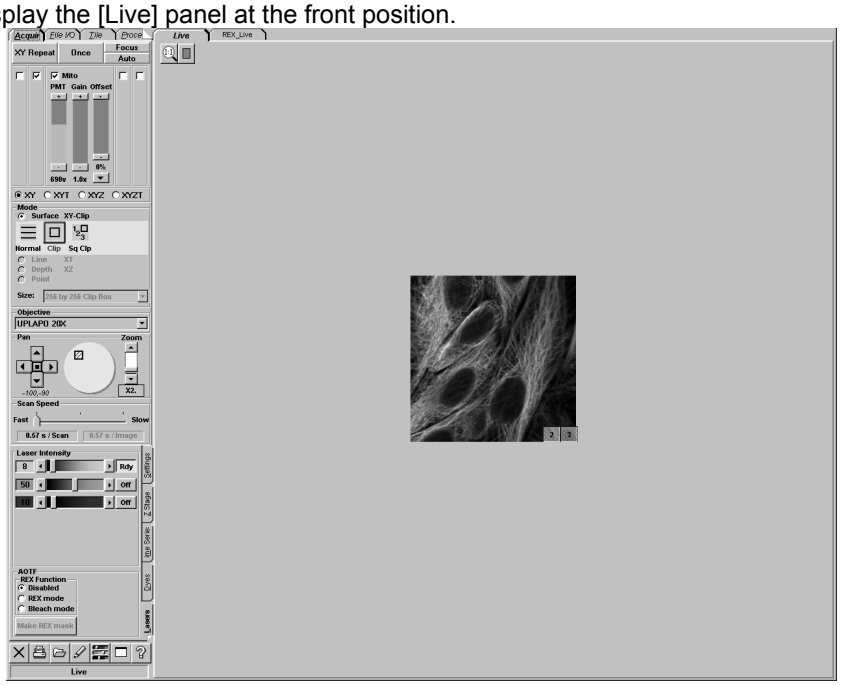


NOTE **In the REX and Bleach mode, image acquisition in the Focus mode, fast scan, and image acquisition in the Line mode (Normal, Slant, and Free) can not be performed.**

NOTE **The specimen in the images carried in this section is not for FRAP, but for the user's manual.**

2-2-12-1 Making REX Mask File

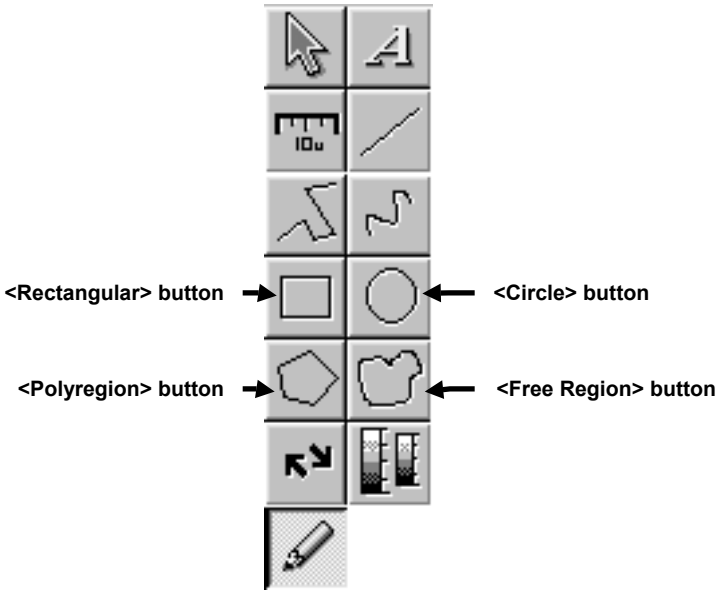
1. Display the [Live] panel at the front position.



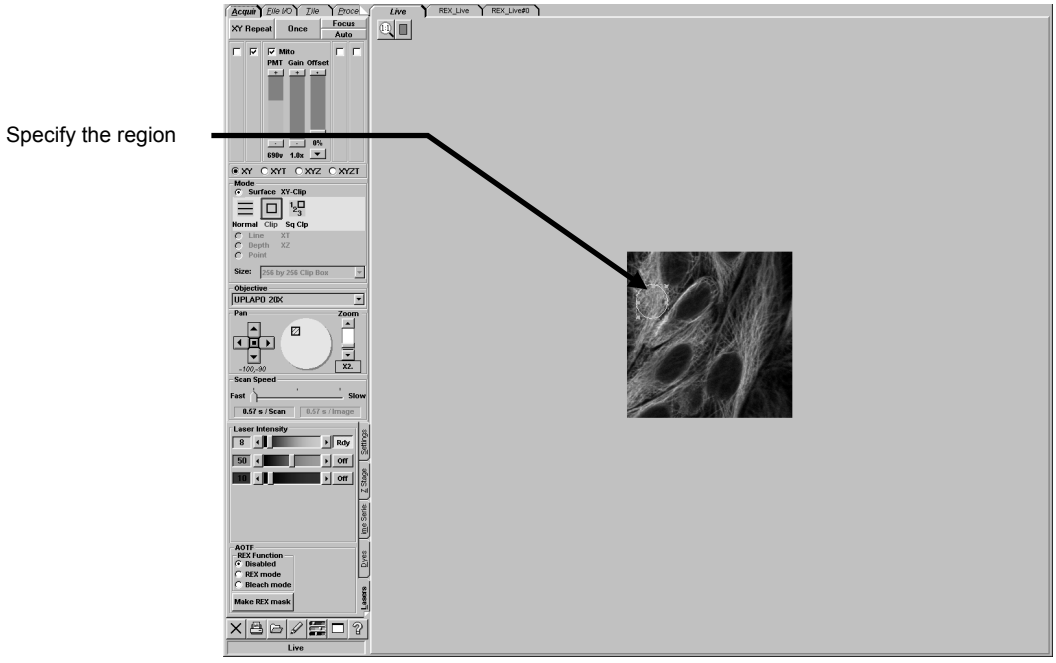
<Annotate> button

2. Select the <Annotate> button in the tool bar.

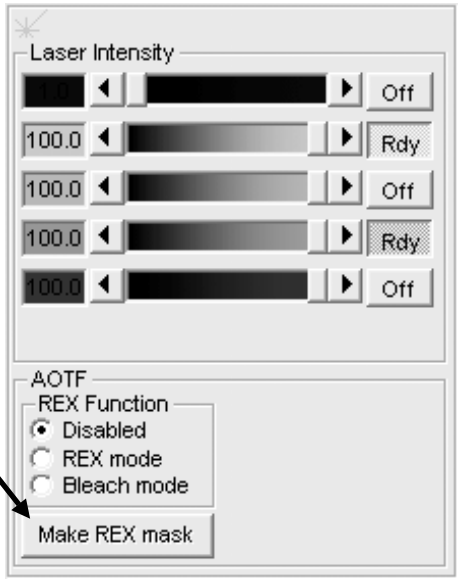
3. In the list of buttons displayed as shown below, select the <Rectangular>, <Circle>, <Polyregion>, or <Free region> button. And drag on the image to specify the region of laser excitation.



TIP In order to specify two or more regions, click the mouse on or inside the region with holding down the SHIFT key after a region is specified.



4. From the page tabs on the bottom right of the [Acquire] panel, select the [Lasers] sub-panel.

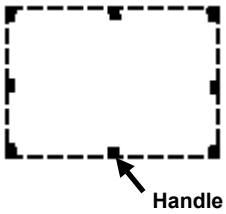


<Make REX mask> button
Makes the REX mask file of the specified region.

Fig. 2-26 [Lasers] sub-panel

TIP

When the <Make REX mask> button is displayed in gray, click and select the specified region on the [Live] panel. When two or more regions are specified, select all the regions to be masked and let the handles be displayed around each region.

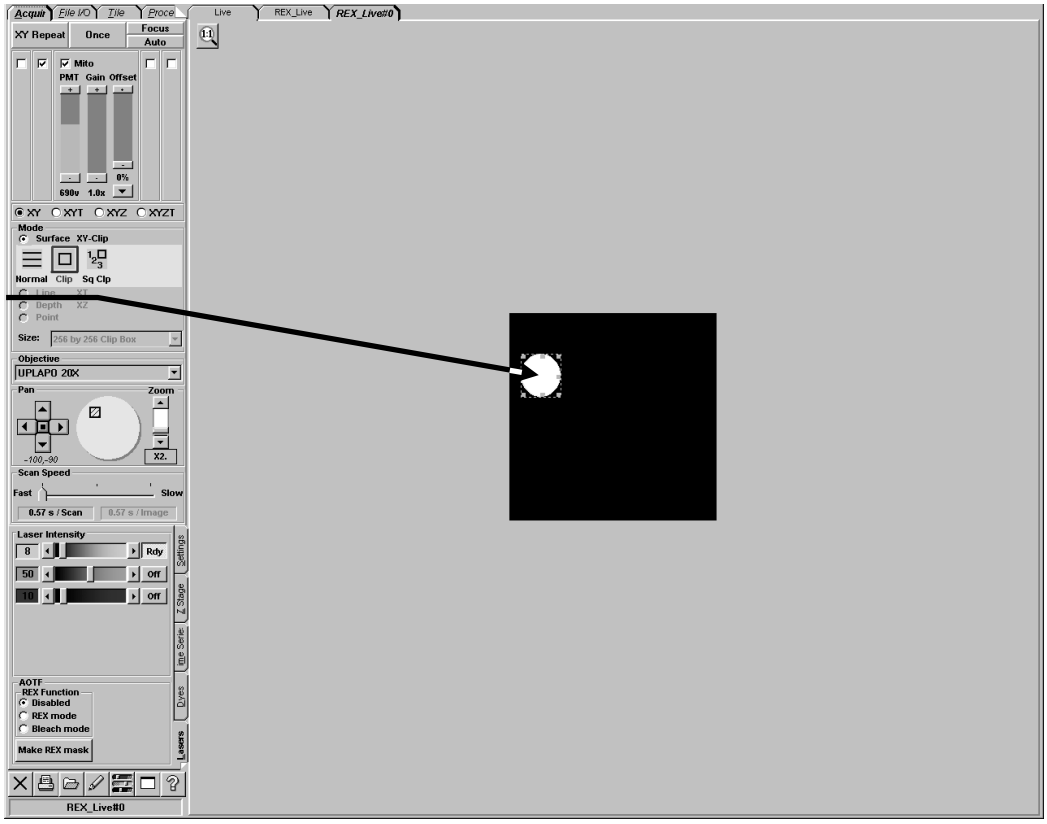


<Make REX mask> button

5. Select the <Make REX mask> button.

   The [REX Live] panel (the REX mask file) is made in the image window.

Make the REX mask file which masks the region outside of the specified region.



TIP

When the [REX Live] panel has already been created on the software, A panel is continuously created after [REX_Live0#].

TIP

The REX mask file can be saved.

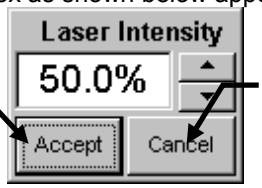
The saving procedure is completely the same as that of a display.

For details, see section 2-3-1-2, “Saving a Display" in this manual.

6. In order to adjust the laser intensity for every region, right-click the mouse on the white region on the image window where the REX mask file is displayed. The pop-up menu as shown below appears.

7. Select [Set intensity] in the menu.

   The [Laser Intensity] dialog box as shown below appears.



<Accept> button

Accepts the value of laser intensity set in the [Laser Intensity] text box and closes the dialog box.

<Cancel> button

Cancels the value of laser intensity set in the [Laser Intensity] dialog box and closes the dialog box.

8. Set the value for the laser intensity on percentage and click the <Accept> button.

   The brightness of the region is changed according to the value set in the [Laser Intensity] dialog box.

TIP The laser intensity of the REX mask file is set by percentage, up to 100%.

TIP The region becomes brighter as the value is close to “100”.

NOTE

In case of using REX function, setting of [Laser Intensity] in [Laser] sub-panel is recommended to set 100%. Otherwise, the actual laser intensity may not be reproducable since the [Laser Intensity] setting affects as followings.

Actual laser intensity = (laser intensity in [Laser Intensity] group box) x (Percentage in the [Laser Intensity] dialog box)

NOTE

When the REX function is used using the visible-light AOTF (FV5-COMBA) and UV-light AOTF (FV5-LUUVAE) simultaneously, the pixels in the visible-light AOTF and those in the UV-light AOTF may be deviated.

2-2-12-2 Example of FRAP experiment

The example of procedure of FRAP experiment using AOTF is described in this section.

In order to perform photobleaching to a specimen, the value of laser intensity is raised to 100% to acquire an image since strong laser intensity is temporarily required. When 100% or more of laser intensity value is required, zoom magnification is gathered to acquire an image. (It is because the square power of zoom magnification per time can be secured.) Moreover, using the objective of larger NA concentrates the laser irradiation spot in Z direction, and stronger laser can be irradiated.
Therefore, a preliminary experiment is required to set up the laser intensity, the objective, the zoom ration of the REX mask file according to a specimen.

1 Preliminary Experiment

1. Set the laser intensity, the objective, and the zoom ration in the [Acquire] panel so that they are suitable for a specimen.

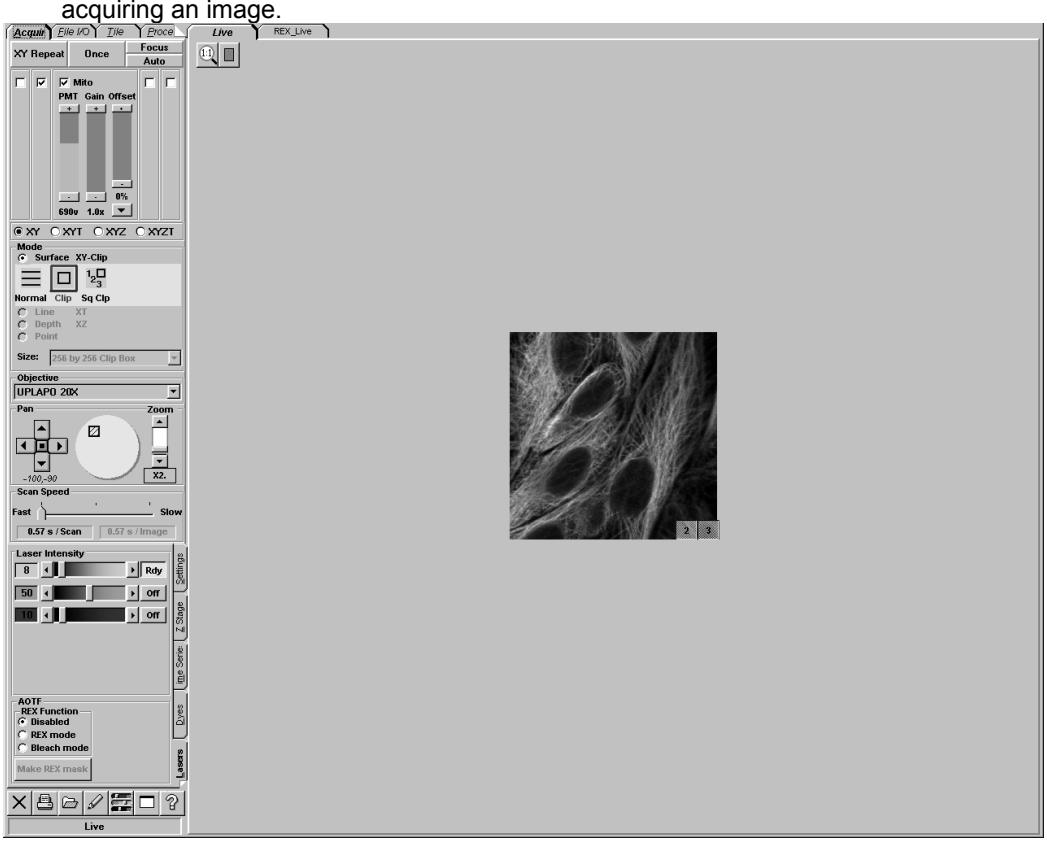
2 Acquiring Image in XY mode

1. Confirm that [XY-Norm] is displayed in the [Mode] group box in the [Acquire] panel.

2. Select the [XY] option button in the [Acquire] panel.

3. Select the <Once> button in the [Acquire] panel and acquire an image.

   If necessary, set the range for image acquisition with clip scan, for example, after acquiring an image.



3 Making REX Mask File

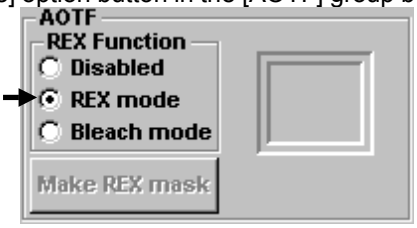
See section 2-2-12-1, “Making REX Mask File” for the procedure to make the REX mask file.

If the setup of the region of laser excitation remains after making the REX mask file, the intensity change of the region can be observed in the TIME COURSE software (optional).
See the “FV-TIEMPO TIME COURSE software User’s manual” for details of the TIME COURSE software.

4 Selecting REX Mask File and Setting the Laser ON/OFF

Set the REX mask file in the REX mode and Bleach mode, and the laser ON/OFF respectively.

1. Select the [REX mode] option button in the [AOTF] group box in the [Lasers] sub-panel.



2. A frame to specify the REX mask file appears on the right side of the [REX mode] option button. Right-click the mouse inside the frame to display the pop-up menu as shown below.

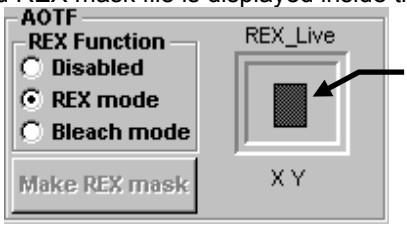
   Select the image to be masked in the menu.

TIP

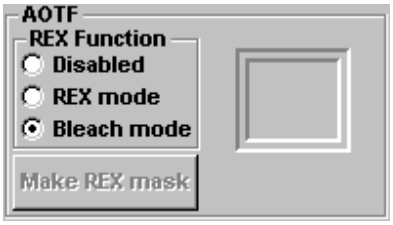
In order to use the already saved file as a REX mask file, open the image beforehand.

The opening method of the REX mask file is completely the same as that of an image. For details, see section 2-3-2, “Opening Previously Saved Images” in this manual.

3. The icon of the selected REX mask file is displayed inside the frame.


AOTF
REX Function
Disabled
REX mode
Bleach mode
Make REX mask
REX_Live
X Y
The icon of the selected REX mask file is displayed inside the frame. And the file name and the observation mode are also displayed above and under the frame.

4. Select the [Bleach mode] option button in the [AOTF] group box of the [Laser] sub-panel.



5. A frame to specify the REX mask file appears on the right side of the [Bleach mode] option button. Right-click the mouse inside the frame to display the pop-up menu as shown below.

   Select the image to be masked in the menu.

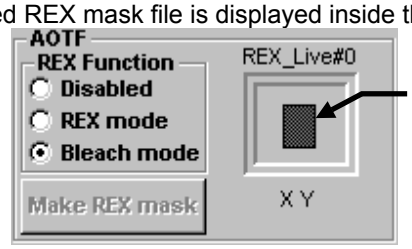
The REX mask file displayed in the image window is pop-up displayed.

TIP

In order to use the already saved file as a REX mask file, open the image beforehand.

The opening method of the REX mask file is completely the same as that of an image. For details, see section 2-3-2, “Opening Previously Saved Images” in this manual.

6. The icon of the selected REX mask file is displayed inside the frame.



The icon of the selected REX mask file is displayed inside the frame. And the file name and the observation mode are also displayed above and under the frame.

7. Set each laser ON/OFF, and their intensity.

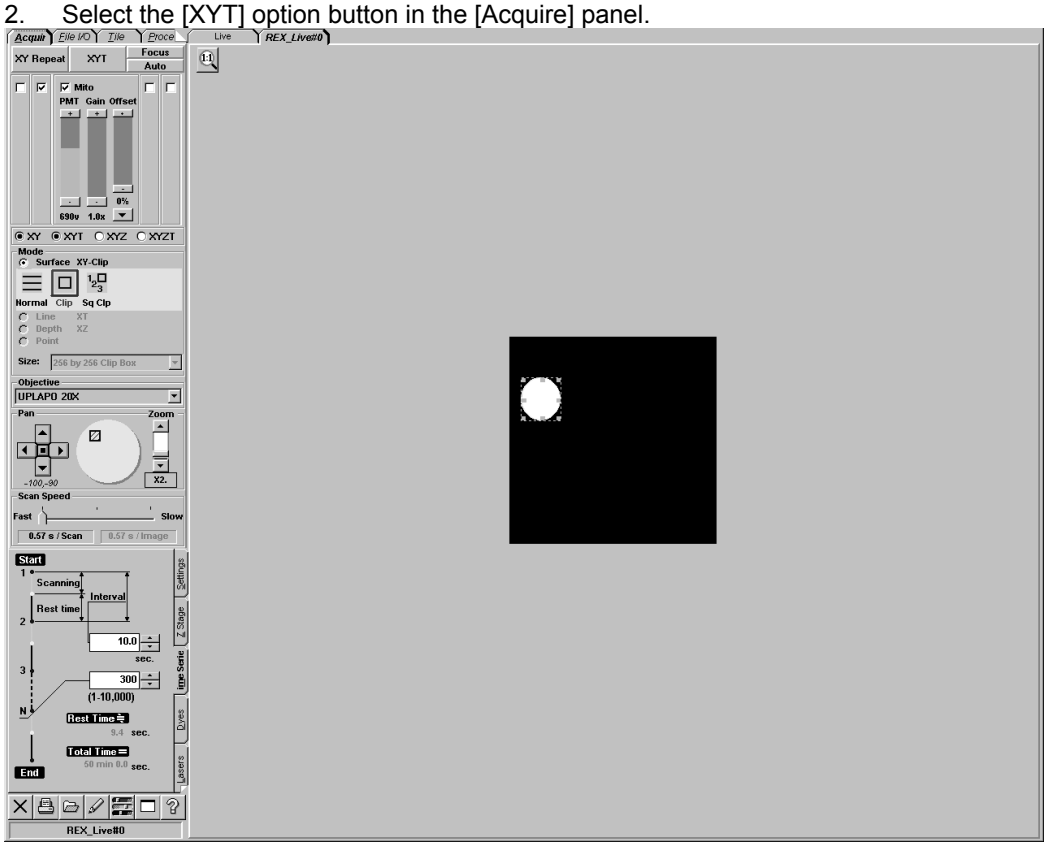
   If necessary, use the [Laser Intensity] dialog box in the [Lasers] sub-panel to set the laser ON/OFF.

   And the value set in the [Laser Intensity] dialog box can be obtained apart from the value set in the Disable/REX mode.

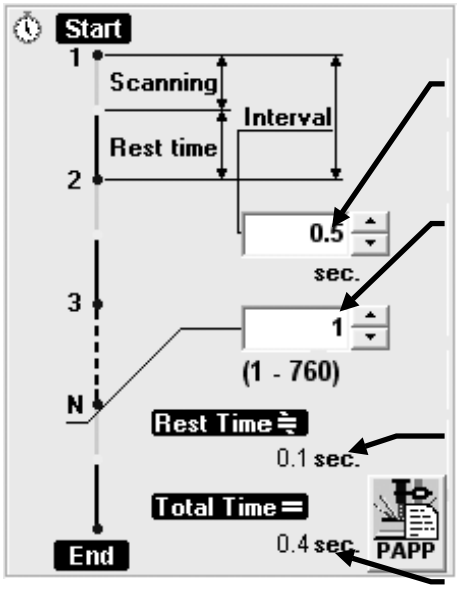
5 Setting the XYT Observation mode

1. Confirm that [XY-Norm] is displayed in the [Mode] group box in the [Acquire] panel.

2. Select the [XYT] option button in the [Acquire] panel.



3. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. The panel as shown below appears.



[Interval] text box

Set the interval with the < ▲ > and< ▼ > buttons. Key entry is also acceptable.

[N] text box

Set the number of scans with the < ▲ > and < ▼ > buttons. Key entry is also acceptable.

Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

[Rest Time] text box

Shows the time after the end of an image acquisition until the start of next acquisition

[Total Time] text box

Shows the time required to complete image acquisition.

Fig. 2-27 [Time Series] sub-panel

4. Set the interval time to “10” using the < ▲ > and < ▼ > buttons in the [Interval] text box. Change the interval time corresponding to the specimen.

5. Set the number of scans to “30” using the < ▲ > and < ▼ > buttons in the [N] text box in the [Time Series] sub-panel. Change the number of scans corresponding to the specimen.

6. Confirm that the time for image acquisition is set to 5 minutes (or 300 seconds) in the [Total Time] text box. The time for image acquisition differs according to the interval and the number of scans.

6 Setting the TIME COURSE Software (optional)

Setting of the real time graph is required when you use the TIME COURSE software.

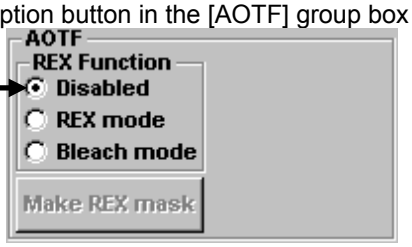
See the “FV-TIEMPO TIME COURSE software User’s manual” for details of the TIME COURSE software.

7 Acquiring Data

The experiment data before and after photobleaching of certain region can be obtained after performing an ordinary image acquisition in the first scan, an image acquisition in the Bleach mode in the second scan , and an ordinary image acquisition in the third scan. When the REX mode is selected in the third scan, the data of the region other than the specified region can not be obtained since the laser is irradiated only to the specified region. However, the REX mode is effective to prevent fading of the obtained data.

1. Select the [Disabled] option button in the [AOTF] group box in the [Lasers] sub-panel.

[Disabled] option button

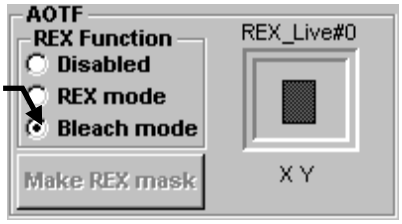


<XYT> button

2. Click the <XYT> button to start image acquisition.

3. Select the [Bleach mode] option button in the [AOTF] group box in the [Lasers] sub-panel.

[Bleach mode] option button

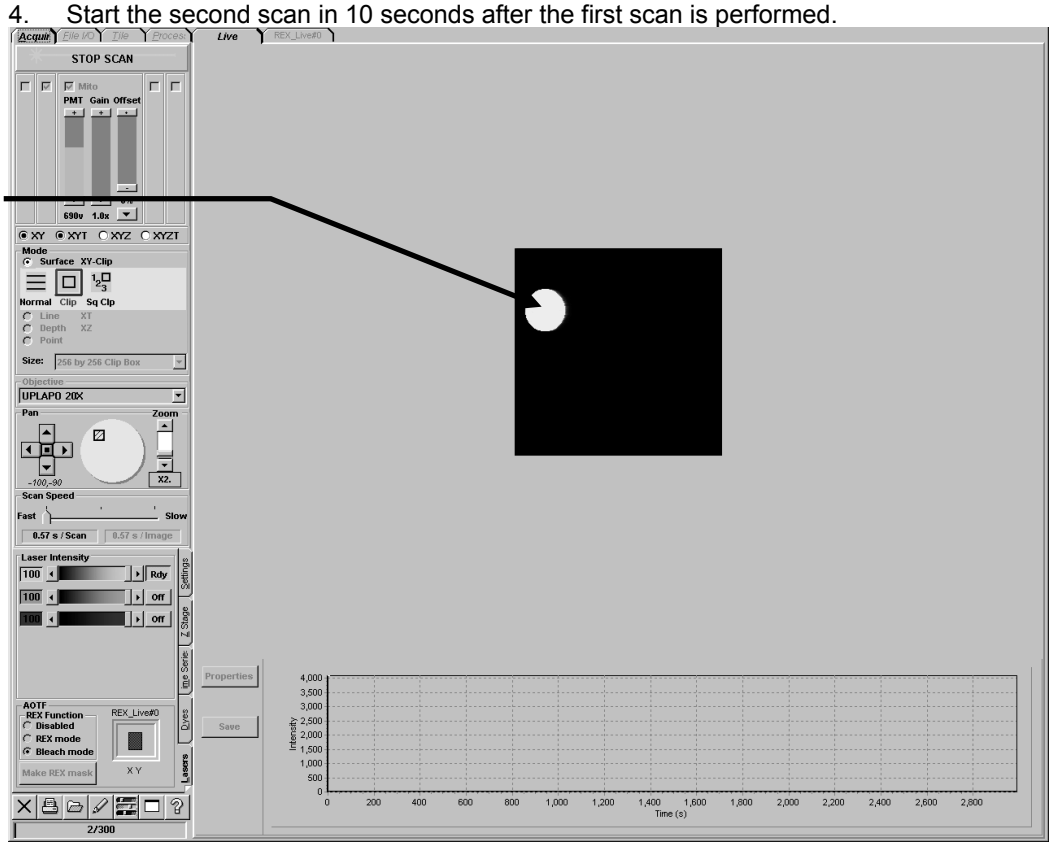


NOTE **Select the [Bleach mode] option button within 10 seconds before the next scanning is started.**

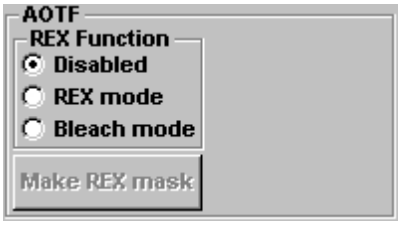
NOTE **In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed. however, the real time graph is not plotted. Please be aware this before using.**

4. Start the second scan in 10 seconds after the first scan is performed.

The laser of strong intensity is irradiated to the specified region.



5. Select the [Disabled] or [REX mode] option button in the [AOTF] group box in the [Lasers] sub-panel.



(The [Disabled] option button is selected.)

NOTE

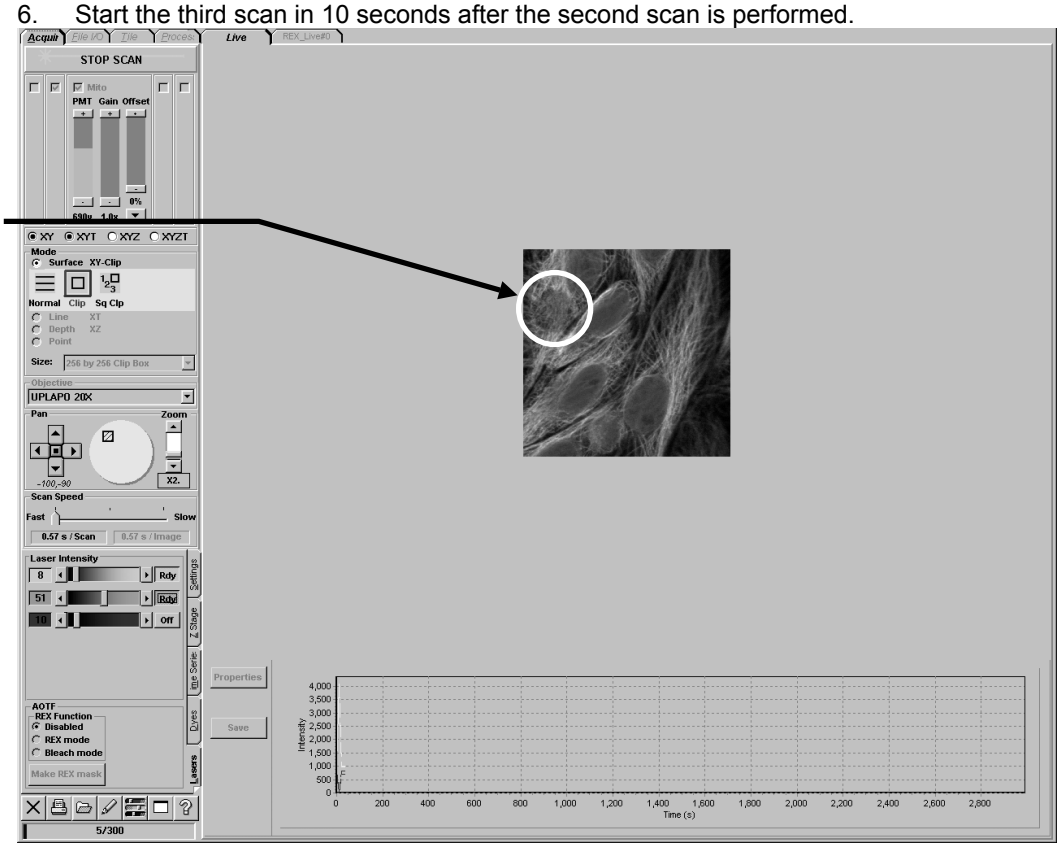
Select the the [Disabled] or [REX mode] option button within 10 seconds before the next scanning is started.

NOTE

In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed, however, the real time graph is not plotted. Please be aware this before using.

6. Start the third scan in 10 seconds after the second scan is performed.

The image of the specimen is fading only where the laser is irradiated.



(The [Disabled] option button is selected.)

7. Start the forth and subsequent scan in 10 seconds after the third scan is performed.

   The image of the photobleached specimen is acquired.

NOTE

When performing the forth or subsequent scan, be sure to switch the [REX mode] and [Bleach mode] between scans.

NOTE

In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed, however, the real time graph is not plotted. Please be aware this before using.

2-2-13 Notes for image acquisition

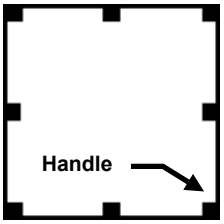
2-2-13-1 Memory of setting information for scanning region

Frame with handle described in 2-2-5 Clip Scan, Frame with hand described in 2-2-6 Zoom-in Scan, Straight line with handle described in 2-2-9, Cross line cursor described in 2-2-10 Point Scan are all called Scanning Region Setting - ROI (Region Of Interest). This Scanning Region Setting – ROI holds the setting information described below when scanning is executed; and the information appears in the same state when each scan mode is selected next time.

·Angle

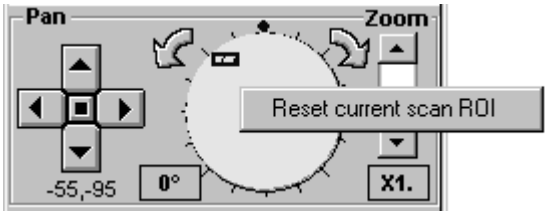
·Profile

·Position



ROI

When default setting information is desired,



click (mouse right) over circle region in [Pan Zoom] group box.

As [Reset current scan ROI] menu appears, select it.

The ROI selected at this time is returned to default setting information.

NOTE **When size of image acquisition is changed, all ROI setting information would be initialized.**

2-3 Saving, Opening and Shredding Images

Use the [File I/O] panel to save, open or shred an image.

Display the [File I/O] panel at the front.

NOTE

The file name has to be within 120 characters including the path for the file.

In case of AVI, the file name is under 250 characters.

<Up> button

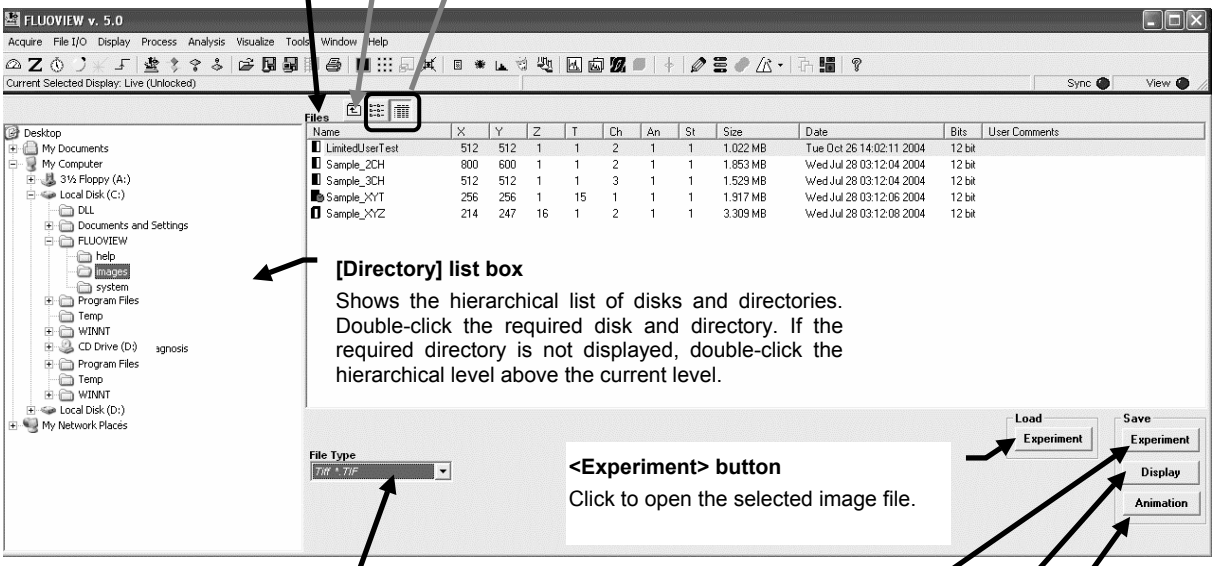
Click to move to the directory above the current directory in the [Files] list box.

[Files] list box

Lists the image files of the type selected with the [File Type] drop-down list and their acquisition parameters. Select the file to be opened from this list. The files in the list can be viewed by scrolling.

<List> and <Details> buttons

These buttons switch the [Files] list box display format. Clicking the <List> button displays only the file names. Clicking the <Details> button displays the file names together with the information (number of channels, comment, etc.) of each file.


FLUOVIEW v. 5.0
Acquire File I/O Display Process Analysis Visualize Tools Window Help
Current Selected Display: Live (Unlocked)
Sync
View
Files
Name X Y Z T Ch An St Size Date Bits User Comments
LimitedUserTest 512 512 1 1 2 1 1 1.022 MB Tue Oct 26 14:02:11 2004 12 bit
Sample_2CH 800 600 1 1 2 1 1 1.853 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_3CH 512 512 1 1 3 1 1 1.529 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_XYT 256 256 1 15 1 1 1 1.917 MB Wed Jul 28 03:12:06 2004 12 bit
Sample_XYZ 214 247 16 1 2 1 1 3.309 MB Wed Jul 28 03:12:08 2004 12 bit
Desktop
My Documents
My Computer
3½ Floppy (A:)
Local Disk (C:)
DLL
Documents and Settings
FLUOVIEW
help
Images
system
Program Files
Temp
WINNT
CD Drive (D:)
Program Files
Temp
WINNT
Local Disk (D:)
My Network Places
File Type
Load
Experiment
Save
Experiment
Display
Animation

[Directory] list box

Shows the hierarchical list of disks and directories. Double-click the required disk and directory. If the required directory is not displayed, double-click the hierarchical level above the current level.

<Experiment> button

Click to open the selected image file.

[File Type] drop-down list

Selects the type of the files to be displayed in the [Files] list box.

<Experiment> button

Saves the image displayed in the image window together with its file name.

< Display> button

Saves the image displayed in the image window as if a hardcopyed image.

<Animation> button

Saves the animation image displayed in the image window.

Fig. 2-28 [File I/O] Panel

<Image Icons>

Images are represented by icons which can also identify the observation modes used when acquiring them.

The icon of the selected image (image in the image window) is displayed in the frame at the top of the function panel such as the [File I/O] panel.

In the [File I/O] panel, image icons are also displayed in the [Icon] field in the [Files] list box.

TIP In all observation modes, the icons for 3 or more channels are identical.

Image Icon	Significance
	XZ observation
	XZ observation, 2-channel mode
	Xt observation
	Xt observation, 2-channel mode
	XZT observation
	XZT observation, 2-channel mode
	XY observation
	XY observation, 2-channel mode
	XYt observation
	XYt observation, 2-channel mode
	XYZ observation
	XYZ observation, 2-channel mode
	XYZt observation
	XYZt observation, 2-channel mode
	Point Scan
	Animation image
	Stereo 3D image: Image to be viewed with color eyeglasses.
	3 or more channels

<[Files] List Box>

<Details> button

<List> button

The information displayed in the list box can be changed.

To display all information, click the <Details> button above the [Files] list box to broaden it.

To return to the display of icons and file names only, click the <List> button.

Icon:
Icons showing the image observation modes.

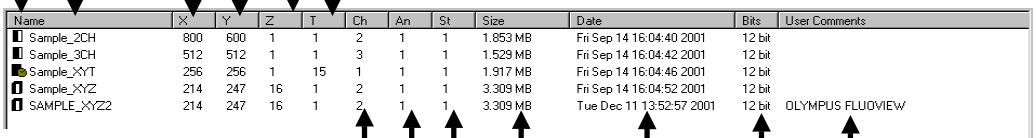
Name:
Image file names.

X:
Image sizes in the X-direction.

Y:
Image sizes in the Y-direction.

Z:
Number of images acquired in the Z-direction.

T:
Number of images acquired over time.



Name	X	Y	Z	T	Ch	An	St	Size	Date	Bits	User Comments
Sample_2CH	800	600	1	1	2	1	1	1.853 MB	Fri Sep 14 16:04:40 2001	12 bit	
Sample_3CH	512	512	1	1	3	1	1	1.529 MB	Fri Sep 14 16:04:42 2001	12 bit	
Sample_XYT	256	256	1	15	1	1	1	1.917 MB	Fri Sep 14 16:04:46 2001	12 bit	
Sample_XYZ	214	247	16	1	2	1	1	3.309 MB	Fri Sep 14 16:04:52 2001	12 bit	
SAMPLE_XYZ2	214	247	16	1	2	1	1	3.309 MB	Tue Dec 11 13:52:57 2001	12 bit	OLYMPUS FLUOVIEW

Ch:
Number of image channels.

An:
Number of images created when constructing a stereo image using the Visualize function.

Stx
"2" is displayed when stereo images (stereo 3D image, or stereo image to be viewed through color eyeglasses) are constructed and saved using the Visualize function.

Size:
Image file sizes.

Date:
Image saving dates.

Bits:
Shows the number of image pixels.

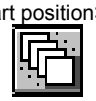
User Comment:
Part of the comment saved with each image.

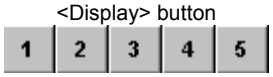
2-3-1 Saving Images

Either a series of images or single image being displayed can be saved.

2-3-1-1 Saving Images As a Series

The displayed images can be saved in a disk as a series image file.





<Display channel switch> buttons

1. Display the image window showing the image to be saved at the front.
2. When there is more than one image to be saved, the <Set start position> and <Set end position> buttons will be displayed on the upper part of the image. Display the image with which the saving should start using the <Display> buttons, then click the <Set start position> button.

   Also set the save end position in the similar way.
3. When the images to be saved are acquired from more than one channel, select whether saving images from more than one channel simultaneously or only a single image from a single channel.

   Use the <Display channel switch> buttons for this selection. The images will be saved according to the conditions of the selected channels.

   Example) When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.

TIP

For the channel switching, see section 2-5-3, “Switching the Displayed Channels”.

4. Click the <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box as shown below appears.

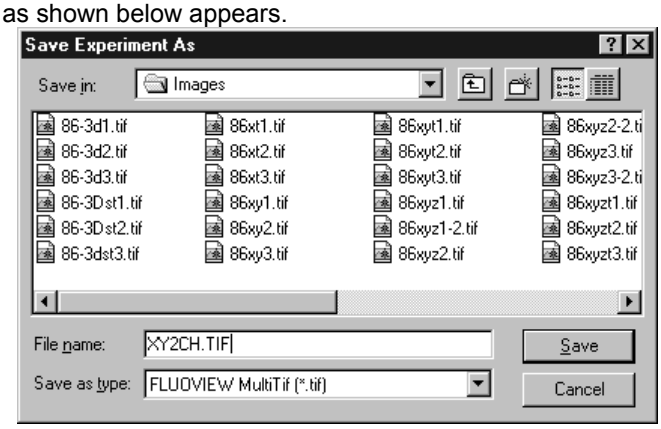


Fig. 2-29 [Save Experiment As] Dialog Box

5. When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.
6. When it is required to change the saved file type, use the [Save as Type:] drop-down list. See section 2-3-1-5, “File Types Available for Save” for details.
7. Enter the file name in the [File Name:] text box.

TIP [Auto Numbering] is enabled image file name appears in [File Name] text box. Then, the image file can store with the numbering just pressing <Save> button.

One Point!

[Auto Numbering] check box moved to [Save Options] dialog box. For details, see section 1-2-2-8, “File I/O Panel” in [FV300/500/1000 Newly Added Functions].

8. Click the <Save> button.

NOTE **If a file with the same name as the entered file name already exists, a dialog box is displayed to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <NO> button and enter another file name.**

One Point!

Live image file is stored to the assigned folder with consecutive numbered file name when [Seq. Number] is selected and [Auto Numbering] is enabled.
([Auto Numbering] check box moved to [Save Options] dialog box. For details, see section 1-2-2-8, “File I/O Panel” in [FV300/500/1000 Newly Added Functions].)

NOTE **[Seq. Number] function is enabled when [Save Experiment As] dialog box appears for not stored Live image is existing.**
Seq. Numbering function is disabled once after the [Save Experiment As] setting is changed.

2-3-1-2 Saving a Display

The displayed images can be hardcopied and saved in a disk. This method is used when it is required to use a FLUOVIEW image in another application.

1. Display the image window showing the image to be saved at the front.

<Display> button

2. When there is more than one image to be saved, display the image to be saved using the <Display> buttons.

3. Click the <Display> button in the [Save] group box. The [Save Experiment As] dialog box as shown below appears.

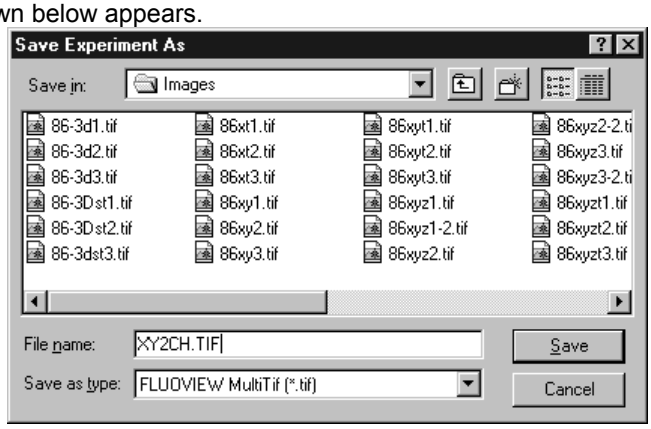


Fig. 2-30 [Save Experiment As] Dialog Box

4. When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.

5. When it is required to change the saved file type, use the [Save as Type:] drop-down list. See section 2-3-1-4, “File Types Available for Save” for details.

6. Enter the file name in the [File Name:] text box.

7. Click the <Save> button.

NOTE

If a file with the same name as the entered file name already exists, a dialog box is displayed to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <NO> button and enter another file name.

2-3-1-3 Saving Specified Area of Image

Only the specified part of an image can be saved as an image in a disk.

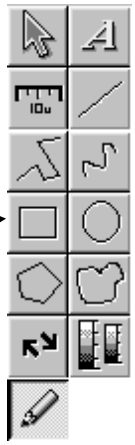
1. Display the image window of the image containing the part to be saved in the front.

2. When the image was acquired through multiple channels, you can select whether the image slices for multiple channels are saved together or the image slice of only 1 channel is saved.
   Use the <display switching> button for the selection. The image will be saved in the channel condition as displayed.

TIP For the channel switching, see section 2-5-3, “Switching the Displayed Channels”.

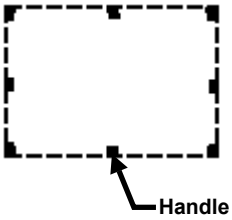
<Annotate> button

3. Specify a desired area in the image. This operation is not necessary if an area has already been specified.
   Click the <Annotate> button in the toolbar. A list of buttons as shown below will appear.



4. Click the <Rectangular> button in this displayed buttons.

5. Specify the area to be saved in the image in the image window.
   The area is displayed on the image with the handles on its frame.
   The area becomes the save target while these handles are displayed.



6. Click the <Annotate> button so that the list of buttons disappear.

7. Click the <Experiment> button in the [Save] group box.
   The [Save Experiment As] dialog box appears.

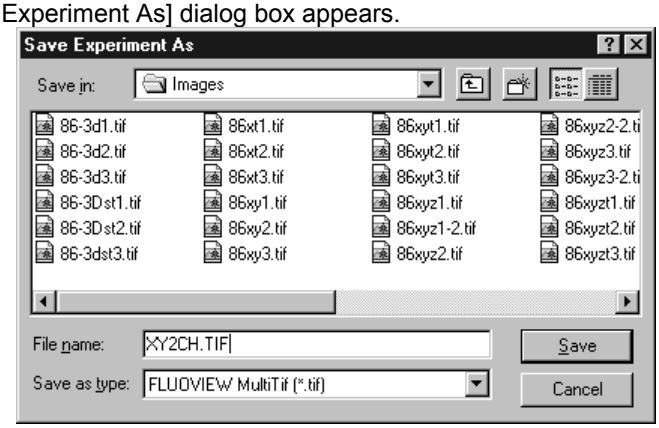


Fig. 2-31 [Save Experiment As] Dialog Box

8. Use the [Save In:] drop-down list if you want to change the save destination drive and directory.

9. Select “FLUOVIEW Multi Tiff” in the [Save as Type:] drop-down list.

TIP It is not possible to save only the specified area of image in the Single TIF or BMP format.

10. Enter the file name in the [File Name:] text box.

11. Click the <Save> button.

12. A dialog box as shown below appears.

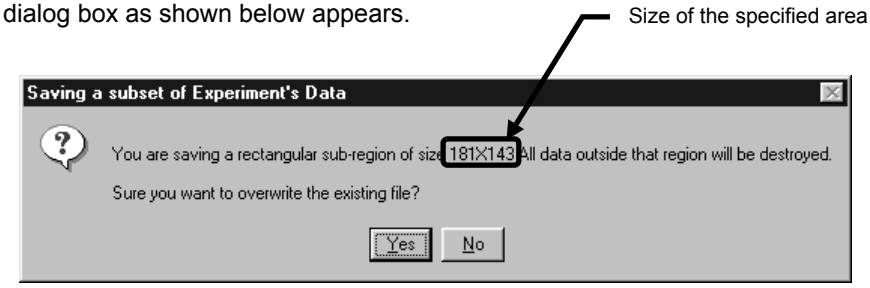

Size of the specified area
Saving a subset of Experiment's Data
You are saving a rectangular sub-region of size 181×143. All data outside that region will be destroyed.
Sure you want to overwrite the existing file?
Yes
No

Fig. 2-32 [Saving a subset of Experiment's Data] Dialog Box

TIP The [Saving a subset of Experiment's Data] dialog box does not appear if no area is specified in the image on the image window.

13. Click the <Yes> button.

2-3-1-4 Saving Animation Images

Transform a created animation image into the AVI file format and save it in the file type which can display the animation image without this software.

1. Create an animation image.
   Refer to “2-9-2 Animation” for details.
2. Display the image window of the created image at the front.

TIP

The AVI file is created to save the animation at the display speed mode selected here. For varying the animation display speed, see section 2-9-1-1, “Changing the Successive Display Speed”.

One Point !

When saving image slices acquired in Z observation, it is recommended to lower the animation display mode by clicking the <Turtle> button. (If image slices are saved with the <Rabbit> button selected, the display speed of the animation speed would become too high.) To change the animation image display speed, see section 2-9-1-1, “Changing the successive display speed”.

3. Select whether saving images from multiple channels simultaneously or only an image from a channel if the images to be saved are acquired from multiple channels.
   Use the <Display Channel Switch> button for this selection. The image is saved according to the condition of the selected channel.

TIP

For the channel switching, see section 2-5-3 “Switching the Displayed Channels”.

<Save Animation>button

4. Click the <Animation> button in the [Save] group box. The [Save Experiment As] dialog box appears as shown below.

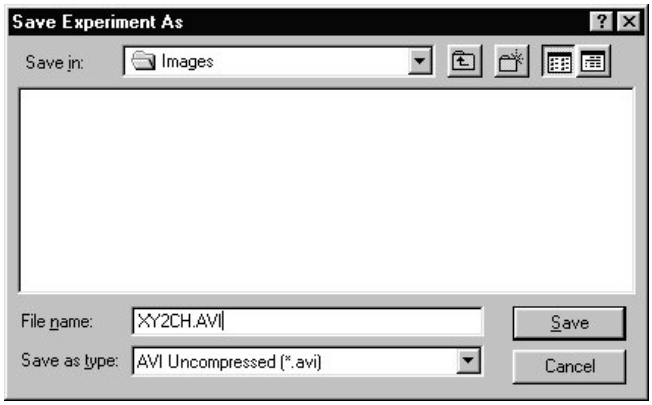


Fig. 2-33 [Save Experiment As] Dialog Box

5. When it is required to change the save destination drive or directory, use the [Save in :] drop-down list.

6. When it is required to change the saved file type, use the [Save as Type :] drop-down list.

TIP

Two saving methods can be selected.
AVI compressed : Saves the AVI file being compressed.
AVI Uncompressed : Saves the AVI file not being compressed.

7. Enter the file name in the [File Name:] text box.

8. Click the <Save> button.

NOTE

If a file with the same name as the entered file name already exists, the dialog box appears to ask if you want to overwrite the existing file. If you do not want to do so, click the <No> button and enter another file name.

2-3-1-5 File Types Available for Save

The file type used for saving image in a file can be selected by the user. See section 2-3-1-1, “Saving Images As a Series” for the operation method.

1. Click the <Experiment> button in the [Save] group box.
2. Select the file type from the [Save as Type:] drop-down list in the [Save Experiment As] dialog box. Three file types are available as detailed below.

• Fluoview Multi Tiff(*.tif):	TIFF format designed for use with FLUOVIEW. Used for image analysis, processing, etc., on FLUOVIEW.
• Single TIF(s) 8-bit(*.tif): • Single TIF(s) 16-bit Gray Scale(*.tif): • Single TIF(s) 24-bit(*.tif):	TIFF (Tagged-Image File Format) is used for image exchange between applications or computers. (Three types including the 8-bit, 16-bit and 24-bit types areavailable.)
• Bitmap 8-bit(*.bmp): • Bitmap 24-bit(*.bmp):	The BMP format is the standard raster format of MS-Windows. (Two types including the 8-bit and 24-bit types are available.)

TIP In the file types above, characters inside ( ) indicate the extension when a file is saved.

NOTE **When saving a display, the file format can be selected from Single TIF(s) 8-bit (*.tif), Single TIF(s) 16-bit (*.tif), Single TIF(s) 24-bit (*.tif), Bitmap 8-bit (*.bmp) and Bitmap 24-bit (*.bmp).**

NOTE **When saving an image obtained by merging more than one channel, use a 24-bit file type.**

NOTE

If you want to save the image with the comment drawn on it as an image, select the file type according to the usage of the image as described below.

Fluoview Multi Tiff: **Select when the image will be analyzed or processed after save. Opening the image also opens the comment, which can be changed or measured as required.**

Single TIF(s): **Select when the image will be handed to another application.**

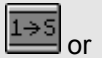
The following table shows the list of image save status by taking the below-mentioned acquisition parameters as an example.

Observation mode:	XYZ observation
Number of channels:	3 channels
Number of acquired images:	4 slices
Save file name:	abcdefgh

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side or Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Fluoview Multi Tiff		The image of only Ch3 is saved. Save file name: abcdefgh.tif	The images of Ch1, Ch2 and Ch3 are saved. Save file name: abcdefgh.tif	The images of Ch1, Ch2 and Ch3 are saved. Save file name: abcdefgh.tif	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	To be saved.
Single TIF(s)	8-bit	12 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif | (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif | (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif | (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.tif | (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	12 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0.tif | (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif | (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif | (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	Not to be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side or Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Single TIF(s)	16-bit	12 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.tif (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	12 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0.tif (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	Not to be saved.

NOTE **All single TIF(s) 16-bit (*.tif) data is saved as gray scale images.**

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side  or Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Single TIF(s)	24-bit	4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif | (Ch1 + Ch2 + Ch3: No. 0) abcdefgh3.tif (Ch1 + Ch2 + Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.tif | (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0V0.tif (Ch1; No. 0) abcdefgh0V3.tif (Ch1; No. 1) abcdefgh0V6.tif (Ch1; No. 2) abcdefgh0V9.tif (Ch1; No. 3) abcdefgh1V1.tif (Ch2; No.0) abcdefgh1V4.tif (Ch2; No.1) abcdefgh1V7.tif (Ch2; No. 2) abcdefgh1V11.tif (Ch2; No.3) abcdefgh2V2.tif (Ch3; No. 0) abcdefgh2V5.tif (Ch3; No. 1) abcdefgh2V8.tif (Ch3; No. 2) abcdefgh2V11.tif (Ch3; No. 3)	The relationship between the saved channels and files are variable depending on the condition of the displayed channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) File No. 0 stores image No. 0, file No. 1 stores the image obtained y accumulating images Nos. 0 and 1,..... The image in the last file is the image obtained by accumulating all of the images. Save file names: abcdefg0;tif | (Image No. 0) abcdefg3;tif (Image Nos. 0 + 1 + 2 + 3)	To be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side or Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Bitmap	8-bit	12 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.bmp | (Ch1: No. 0) abcdefgh3.bmp (Ch1: No. 3) abcdefgh4.bmp | (Ch2: No. 0) abcdefgh7.bmp (Ch2: No. 3) abcdefgh8.bmp | (Ch3: No. 0) abcdefgh11.bmp (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.bmp | (Ch3: No. 0) abcdefgh3.bmp (Ch3: No. 3)	12 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0.bmp | (Ch1: No. 0) abcdefgh3.bmp (Ch1: No. 3) abcdefgh4.bmp | (Ch2: No. 0) abcdefgh7.bmp (Ch2: No. 3) abcdefgh8.bmp | (Ch3: No. 0) abcdefgh11.bmp (Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	Not to be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side or Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Bitmap	24-bit	4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.bmp | (Ch1 + Ch2 + Ch3: No. 0) abcdefgh3.bmp | (Ch1 + Ch2 + Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.bmp | (Ch3: No. 0) abcdefgh3.bmp (Ch3: No. 3)	4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0V0.bmp (Ch1; No. 0) abcdefgh0V3.bmp (Ch1; No. 1) abcdefgh0V6.bmp (Ch1; No. 2) abcdefgh0V9.bmp (Ch1; No. 3) abcdefgh1V1.bmp (Ch2; No.0) abcdefgh1V4.bmp (Ch2; No.1) abcdefgh1V7.bmp (Ch2; No. 2) abcdefgh1V11.bmp (Ch2; No.3) abcdefgh2V2.bmp (Ch3; No. 0) abcdefgh2V5.bmp (Ch3; No. 1) abcdefgh2V8.bmp (Ch3; No. 2) abcdefgh2V11.bmp (Ch3; No. 3)	The relationship between the saved channels and files are variable depending on the condition of the displayed channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) File No. 0 stores image No. 0, file No. 1 stores the image obtained y accumulating images Nos. 0 and 1,..... The image in the last file is the image obtained by accumulating all of the images. Save file names: abcdefgh0;bmp | (Image No. 0) abcdefgh3;bmp (Image Nos. 0 + 1 + 2 + 3)	To be saved.

2-3-2 Opening Previously Saved Images

Image files saved in the disk can be opened as follows.

1. If the image file name that you want to open is not displayed in the [Files] list box, change the drive and/or directory to those containing the desired file using the [Drive] drop-down list and/or [Directory] list box.
2. From the [File Type] drop-down list, select the file type of the files to be listed in the [Files] list box.
3. Click the <Experiment> button in the [Load] group box.

TIP

Other methods are also available for opening a file:

Perform the same operations as steps 1 and 2 above before the following.

- Place the mouse pointer on the desired image file name in the [Files] list box and double-click it.

2-3-3 Shredding Images

Shredding an image refers to removing it from the objects of processing including display. **Shredding does not actually deletes the image saved in the disk**.

<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.

Fig. 2-34 [Experiments in Memory] Dialog Box

<Shredder> button

2. In the [Experiments in Memory] dialog box, select the file name of the image to be shredded and click the <Shredder> button.
   The file can also be shredded by placing the mouse pointer on it and dragging it to the <Shredder> button.

TIP The mouse pointer transforms to an image icon during dragging.

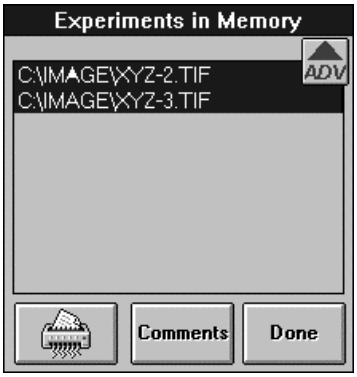
3. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

TIP

Multiple images displayed can be shredded simultaneously.

1. Display the [Experiments in Memory] dialog box while showing multiple images in the image window.
2. Pressing down the Shift key, select multiple image files to be shredded.
3. Click the <Shredder> button.

<Shredder> button



4. Click the <Done> button in the [Experiments in Memory] dialog box to close.

One Point!

[Experiments in Memory] dialog box moved to [Experiment Editor] Function Window.

Select <Experiment Editor> button on tool bar and bring Function Window to appear.

<Experiment Editor> button

2-3-4 Saving Comment Together with Image

<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.

Fig. 2-35 [Experiments in Memory] Dialog Box

One Point!

[Experiments in Memory] dialog box moved to [Experiment Editor] Function Window.

Select <Experiment Editor> button on tool bar and bring Function Window to appear.

<Experiment Editor> button

2. In the [Experiments in Memory] dialog box, select the file name of the image to be saved with comment and click the <Comments> button. The [Image Comments] dialog box appears as shown below.

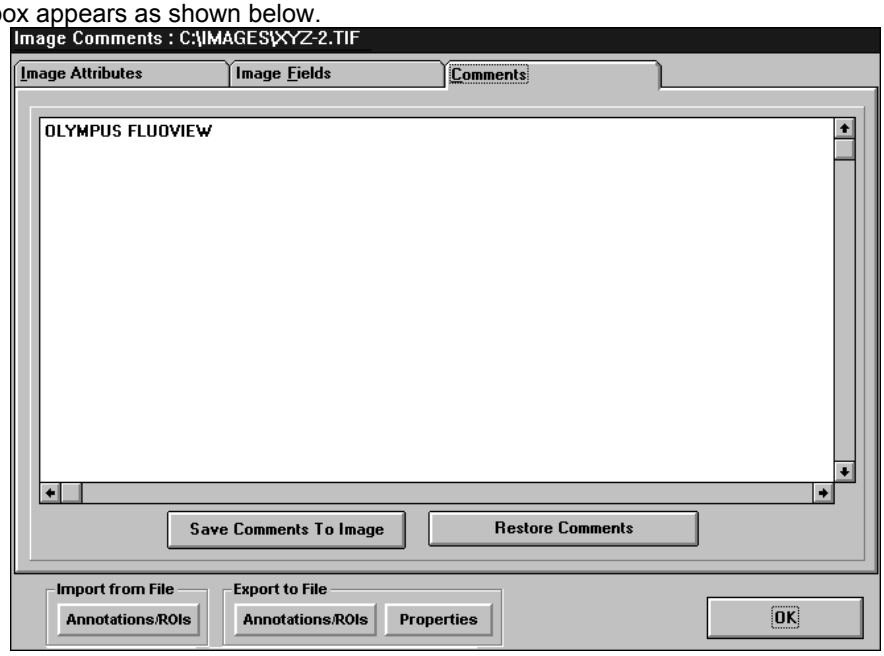


Fig. 2-36 [Image Comments] Dialog Box

3. Display the [Comments] panel at the front.

4. Enter comment from the keyboard.

NOTE **During modification of previously entered commenbmp it is required to restore the originally entered comment, click the <Restore Comments> button. However, once the <Save Comments To Image> button is pressed, the original comment cannot be restored.**

5. Click the <Save Comments To Image> button.

NOTE **At the moment the <Save Comments To Image> button is clicked, the comment in the [Comments] panel is saved simply, but it is not yet saved in the image file. To save the comment in the image file, use the <Save> button in the [File I/O] panel.**

6. Click the <OK> button.

7. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

8. Display the [File I/O] panel at the front.

9. Click the <Experiment> button in the [Save] group box.

TIP

See section 2-3-1, “Saving Images” for details.

One Point!

The [Image Comments] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the image window, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Experiment Properties] from the menu.

2-3-5 Checking the Image Information/Acquisition Parameters

<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.

Fig. 2-37 [Experiments in Memory] Dialog Box

One Point!

[Experiments in Memory] dialog box moved to [Experiment Editor] Function Window.

Select <Experiment Editor> button on tool bar and bring Function Window to appear.

<Experiment Editor> button

2. In the [Experiments in Memory] dialog box, select the file name of the image to check the image information and acquisition parameters and click the <Comments> button.

3. Display the [Image Attributes] panel at the front. The panel as shown below appears, where the information on the selected image is shown.

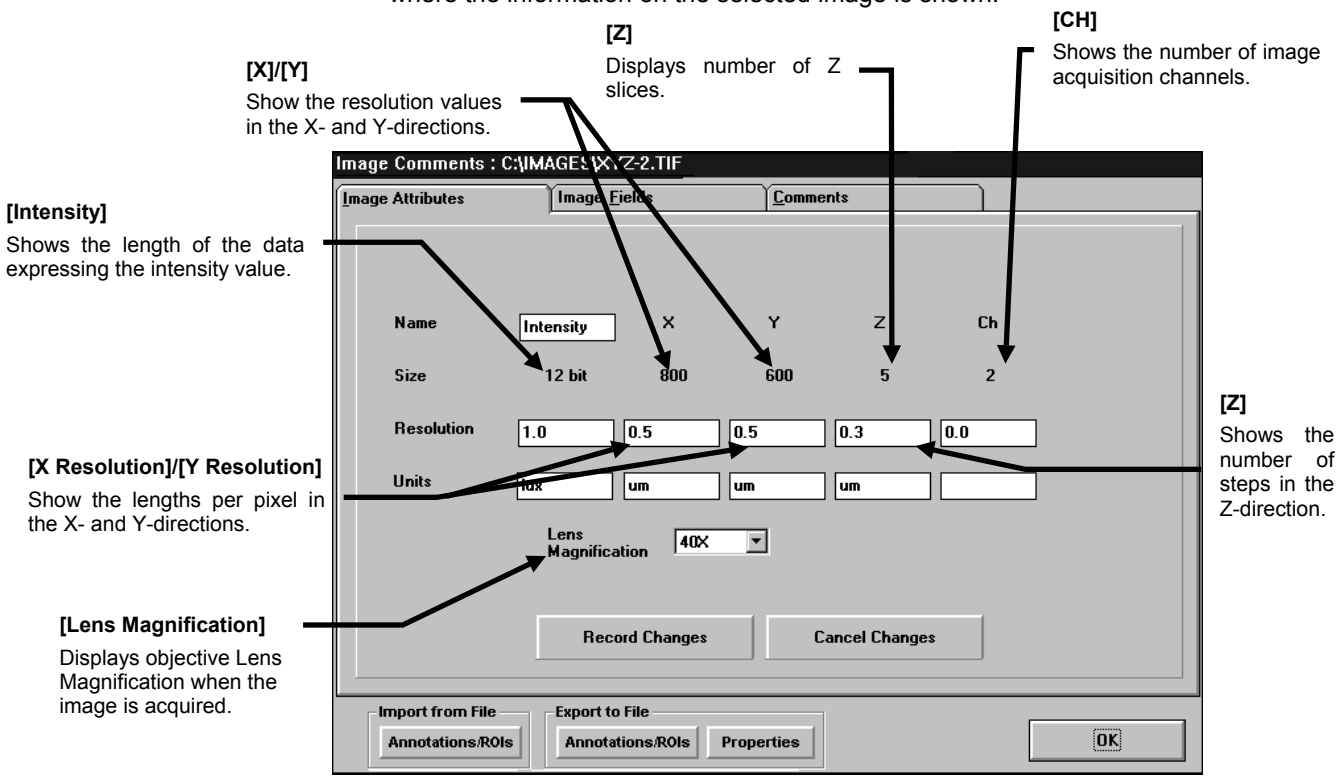


Fig. 2-38 [Image Attributes] Panel

TIP

The [X Size], [Y Size] and [Z Size] text boxes can also be used to change the lengths per image pixel or the number of steps in the Z-direction. These values are used in the scale display and many other analysis operations. When opening and analyzing an image file creased with another application on FLUOVIEW, enter the lengths per image pixel and the number of steps in the Z-direction if these values are known, then click the <Set Attributes> button.

NOTE

During modification of the lengths per image pixel, the number of steps in the Z-direction or the objective setting, if it is required to restore the previous setting, click the <Restore Attributes> button. However, once the <Set Attributes> button is pressed, the original setting cannot be restored.

NOTE **The objective setting can be changed even after having acquired the image. If the objective is not set with the [Acquire] panel before image acquisition, the analysis and measurement results of the image may be erroneous. In such a case, set the objective again.**

NOTE **When the objective setting is changed, the lengths per image pixel will be re-calculated automatically and changed.**

4. Display the [Image Fields] panel at the front. The panel as shown below appears, where the information on the selected image is shown.

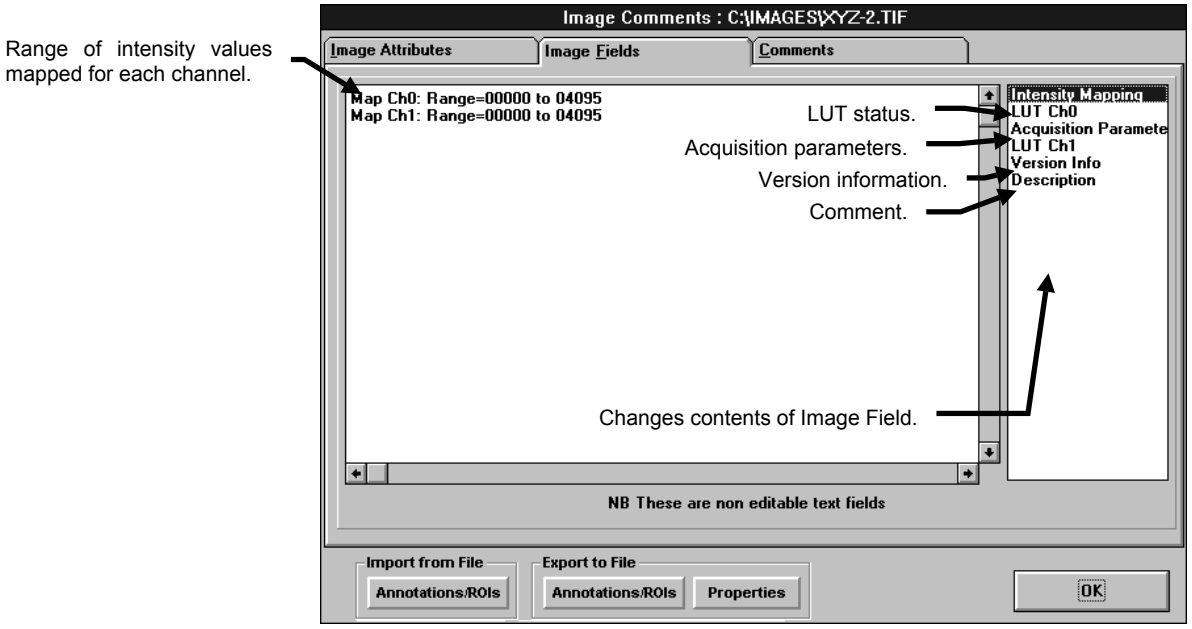
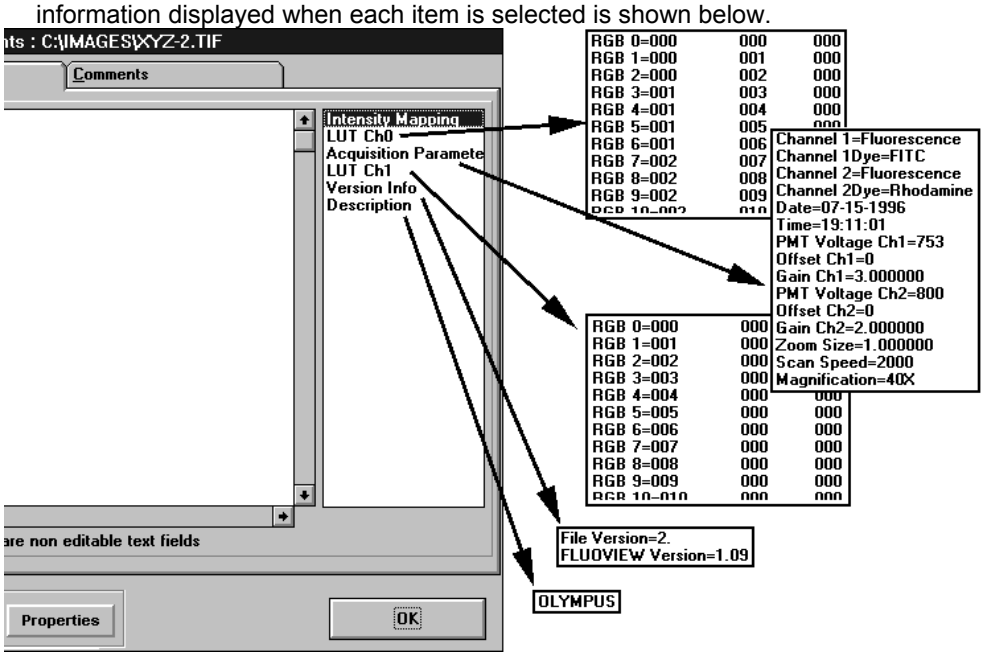


Fig. 2-39 [Image Fields] Panel

5. Select the information items to be checked from the list box on the right. The kind of information displayed when each item is selected is shown below.



6. After checking the information, click the <OK> button.
7. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

One Point!

The [Image Comments] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the image window, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Experiment Properties] from the menu.

2-3-6 Saving the Image Information/Observation Condition

The image information and observation condition can be saved as ASCII text file in a disk.

1. Display the image window of the image to be saved at the front position.

<Experiment List> button

2. Select the <Experiment List> button in the tool bar displayed at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.

Fig. 2-40 [Experiments in Memory] dialog box

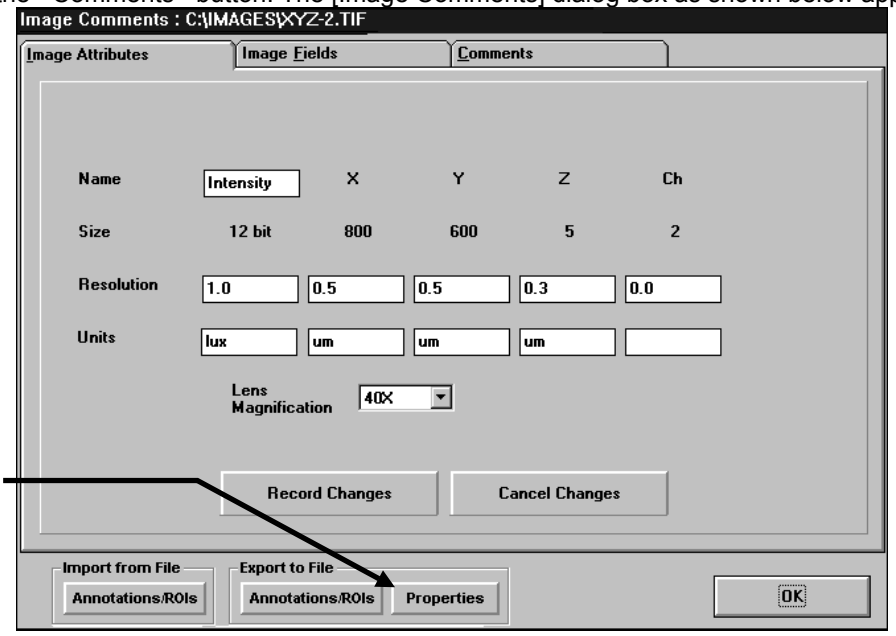
One Point!

[Experiments in Memory] dialog box moved to [Experiment Editor] Function Window.

Select <Experiment Editor> button on tool bar and bring Function Window to appear.

<Experiment Editor> button

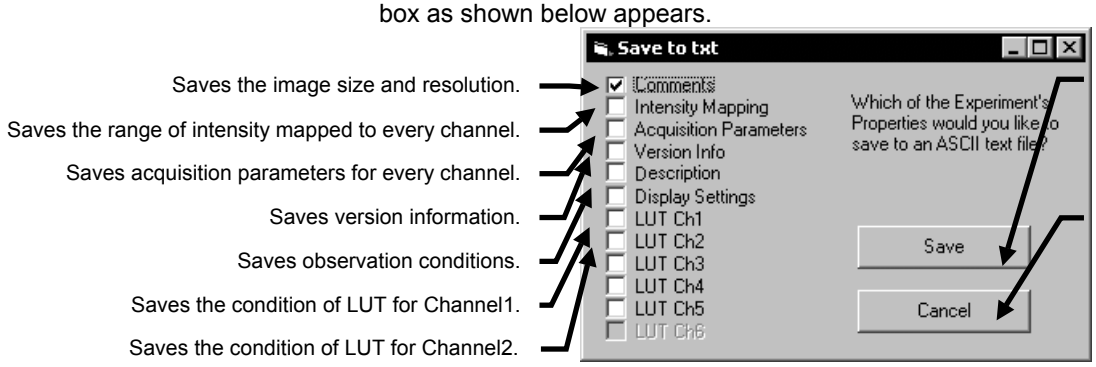
3. In the list in the [Experiments in Memory] dialog box, select the file name including the image whose image information and observation condition are to be saved. And click the <Comments> button. The [Image Comments] dialog box as shown below appears.



<Properties> button in the [Export to File] group box
Saves the image information and observation condition.

Fig. 2-41 [Image Comments] dialog box

4. Select the <Properties> button in the [Export to File] group box. The [Save to txt] dialog box as shown below appears.



Saves the image size and resolution.
Saves the range of intensity mapped to every channel.
Saves acquisition parameters for every channel.
Saves version information.
Saves observation conditions.
Saves the condition of LUT for Channel1.
Saves the condition of LUT for Channel2.

<Save> button
Saves the checked item into the text file.

<Cancel> button
Cancels the checked item and closes the dialog box.

Fig. 2-42 [Save to txt] dialog box

TIP The [LUT Ch1] to [LUT Ch6] check boxes are displayed according to the channel used for image acquisition.

5. Check the check box of the item to be saved.

6. Select the <Save> button. The [Save As ASCII Text] dialog box as shown below appears.

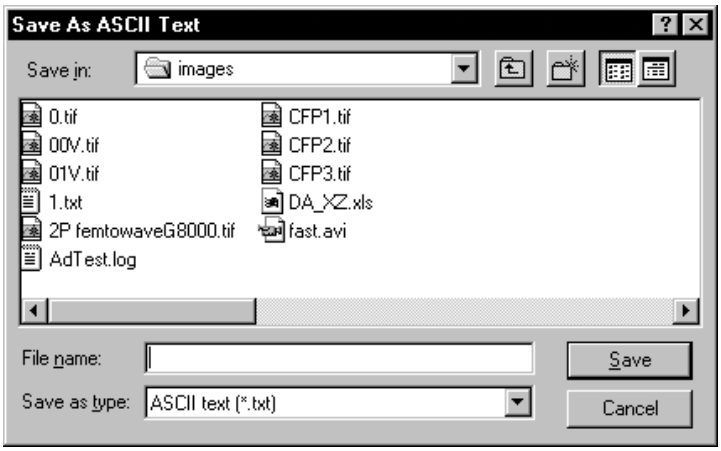


Fig. 2-43 [Save As ASCII Text] dialog box

7. In order to change the save destination drive or directory, use the [Save in:] drop-down list.

8. Confirm that “ASCII text (*.txt)” is selected in the [Save as type:] drop-down list.

9. Enter a file name into the [File Name:] text box.

10. Click the <Save> button.

NOTE **When the text file having the same name as the entered name is already exists, the dialog box appears to ask you to overwrite the existing file or not. When overwriting is not required, click the <NO> button and enter another name to save the file.**

TIP When the image to be saved was acquired through multiple channels, the observation conditions for all channels are saved.

2-3-7 Saving/Reading the Region File

The information on the shape, position, and color of certain region can be saved in a file and they can also be read.

2-3-7-1 Saving the Region File

1. Display the image window of the image including the region to be saved at the front position.

<Experiment List> button

2. Select the <Experiment List> button in the tool bar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.

Fig. 2-44 [Experiments in Memory] dialog box

One Point!

[Experiments in Memory] dialog box moved to [Experiment Editor] Function Window.

Select <Experiment Editor> button on tool bar and bring Function Window to appear.

<Experiment Editor> button

3. Select the image file name including the region to be saved in the [Experiments in Memory] dialog box and click the <Comments> button.

   The [Image Comments] dialog box as shown below appears.

<Annotations/ROIs> button in the [Export to File] group box
Saves the region into a file.

<Annotations/ROIs> button in the [Import from File] group box
Opens the file saving the region.

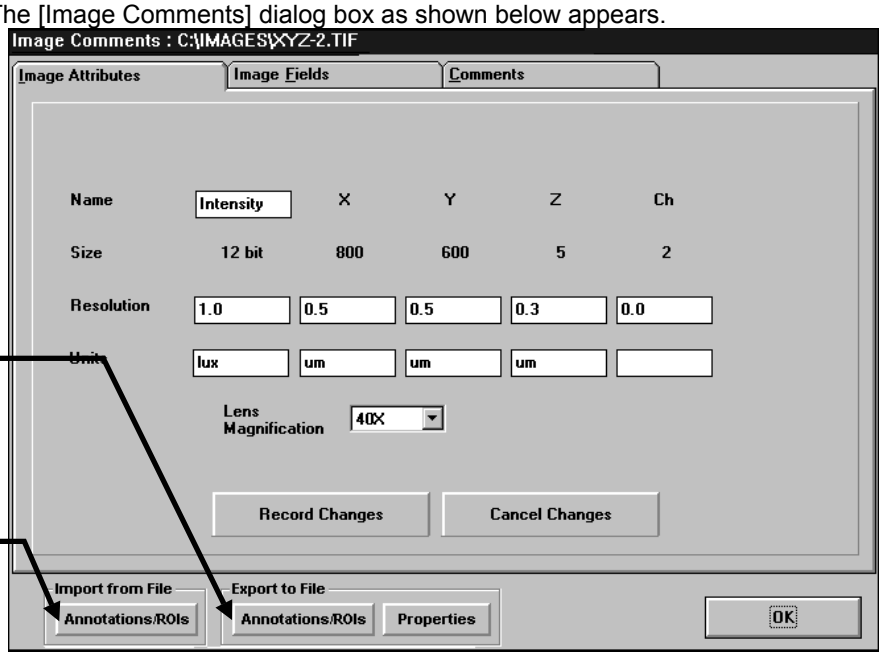


Fig. 2-45 [Image Comments] dialog box

4. Select the <Annotations/ROIs> button in the [Export to File] group box.

   The [Save Experiment As] dialog box as shown below appears.

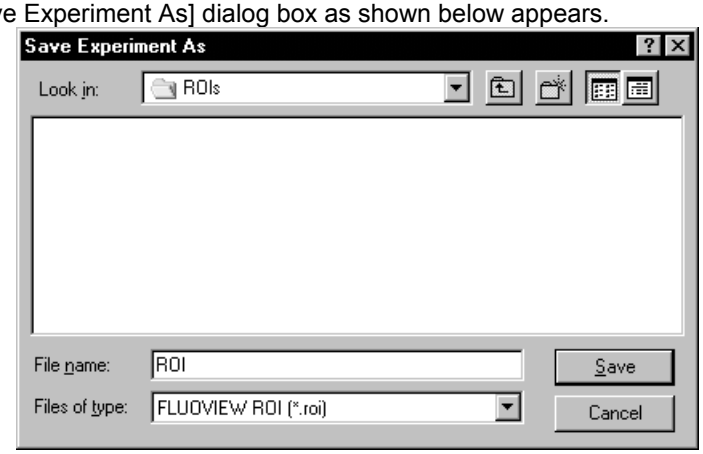


Fig. 2-46 [Save Experiment As] dialog box

5. Select the save destination drive or folder in the [Look in] drop-down list.

6. Select “FLUOVIEW ROI (*.roi)” in the [Files of type] drop-down list.

7. Enter the file name into the [File name] text box and click the <Save> button.

NOTE

When the text file having the same name as the entered name is already exists, the dialog box appears to ask you to overwrite the existing file or not. When overwriting is not required, click the <NO> button and enter another name to save the file.

2-3-7-2 Reading the Region File

1. Display the image window of the image including the region to be read at the front position.

<Experiment List> button

2. Select the <Experiment List> button in the tool bar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.

Fig. 2-47 [Experiments in Memory] dialog box

One Point!

[Experiments in Memory] dialog box moved to [Experiment Editor] Function Window.

Select <Experiment Editor> button on tool bar and bring Function Window to appear.

<Experiment Editor> button

3. In the list in the [Experiments in Memory] dialog box, select the image file name including the region to be read and click the <Comments> button.
   The [Image Comments] dialog box as shown below appears.

<Annotations/ROIs> button in the [Export to File] group box
Saves the region into a file.

<Annotations/ROIs> button in the [Import from File] group box
Reads the file saving the region.

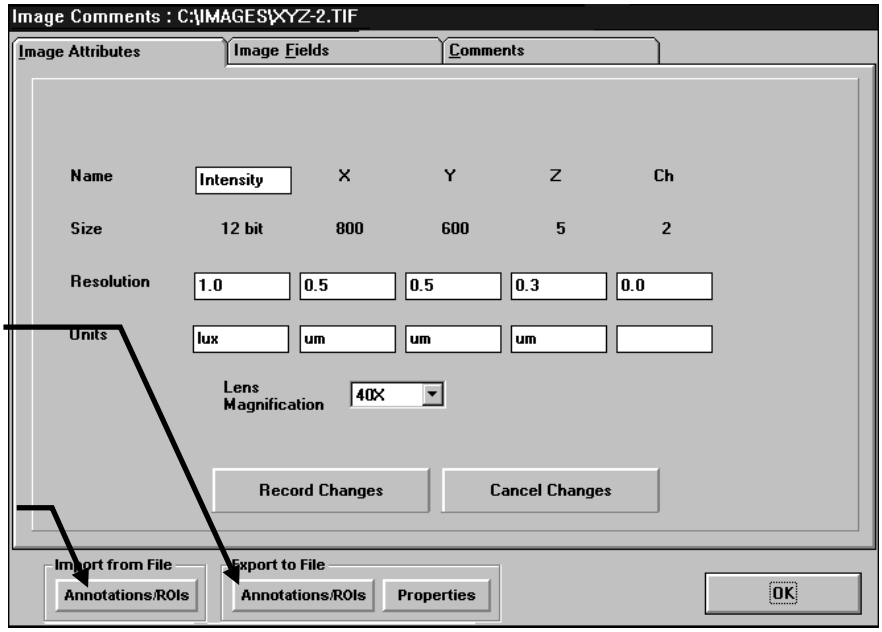


Fig. 2-48 [Image Comments] dialog box

4. Select the <Annotations/ROIs> button in the [Import from File] group box.
   The [Save Experiment As] dialog box as shown below appears.

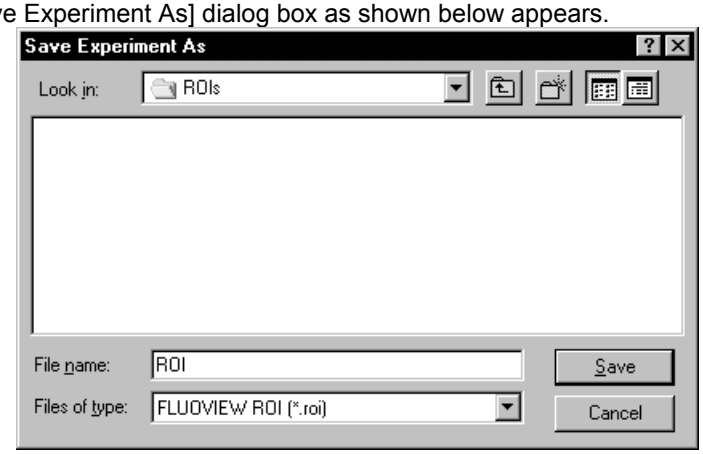


Fig. 2-49 [Save Experiment As] dialog box

5. Select the drive or folder where the file to be read is saved in the [Look in] drop-down list.

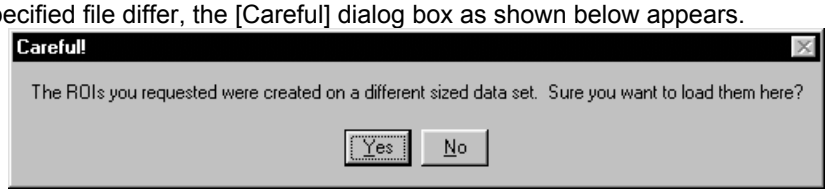
6. In the list below the [Look in] drop-down list, double-click the folder or sub-folder where the data to be read is saved to open.

7. Select “FLUOVIEW ROI (*.roi)” in the [Files of type] drop-down list.

8. Select the file to be read and click the <Open> button.

TIP

When the image size of the file including the region to be read and that of the specified file differ, the [Careful] dialog box as shown below appears.



Clicking the <OK> button reads the file in different image size, whereas clicking the <No> button cancels reading the region file.

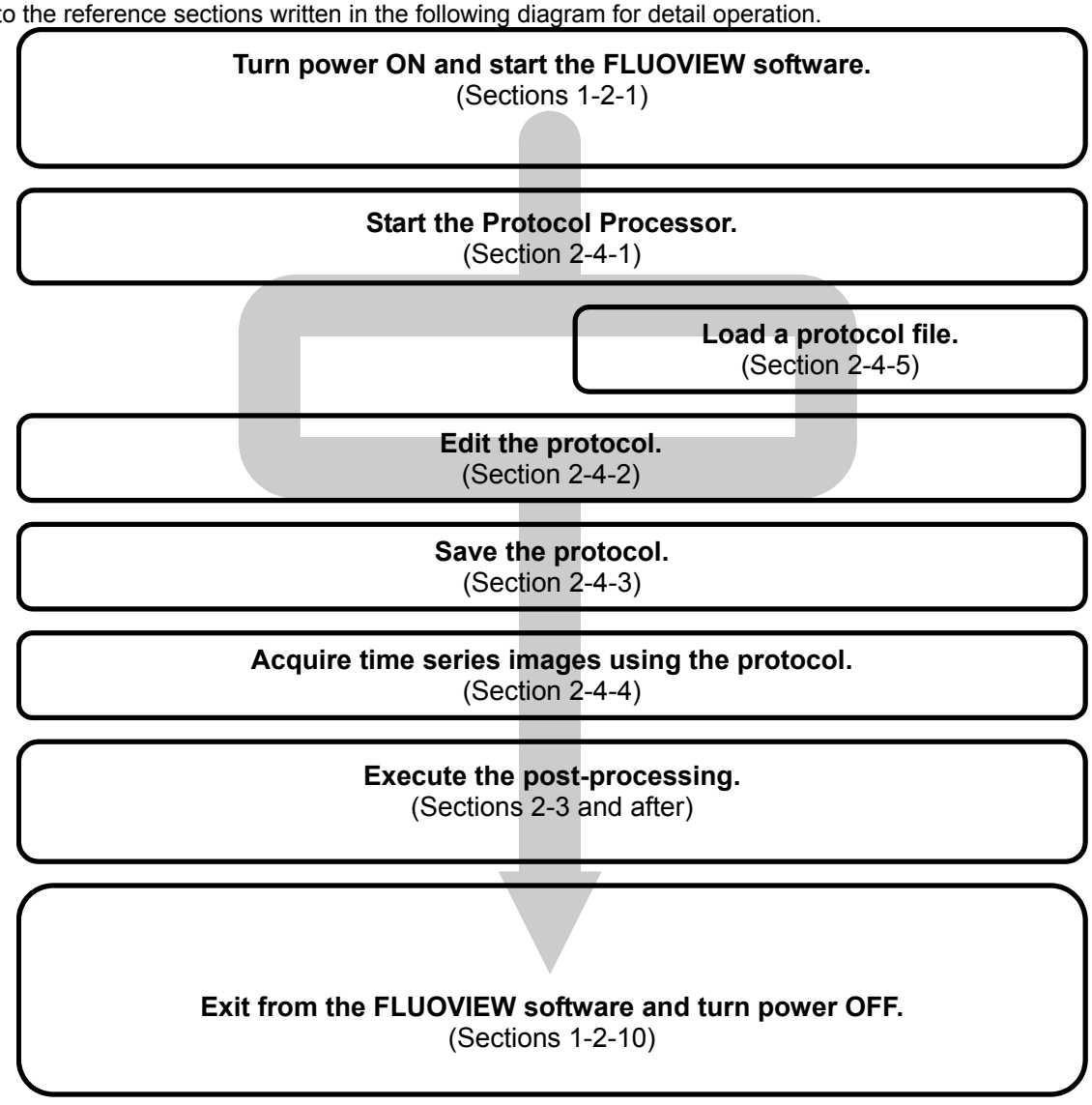
When the region file is read in different image size, the upper left of the image is assumed as reference.

2-4 Protocol processor

The Protocol Processor makes FLUOVIEW image acquisition flexible, especially time series image acquisition. The function enables to create and modify the experiment procedure such as image acquisition condition with interval setting, laser excitation area and its power setting.

The protocol can save and modify to be suitable to new experiment.

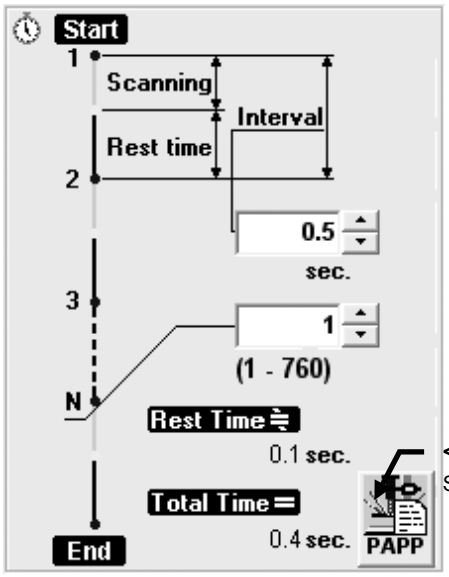
Refer to the reference sections written in the following diagram for detail operation.



The protocol can be edited by Protocol Processor software that comes with the FLUOVIEW software.

2-4-1 Starting the Protocol Processor

1. Starts the Protocol Processor by clicking <PAPP> button in [Time Series] sub-panel of [Acquire] panel.

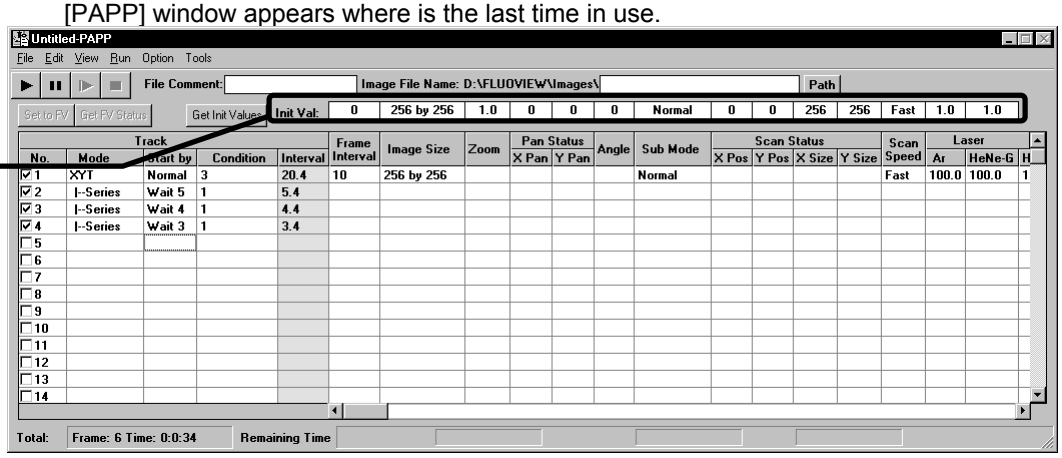


<PAPP> button
Starts the Protocol Processor.

The [Programmable Acquisition Protocol Processor] window appears as shown below. (It is referred to as the [PAPP] window in this manual.)

[PAPP] window appears where is the last time in use.

[Init Val] box
Shows the default values of each parameters.



: <Start> button : Starts the protocol.

:<Break> button :Temporally stops the protocol, it can re-start by pressing <Start> button.

:<Skip> button :Skips to the next Track of the protocol.

:<End> button :Stops the protocol.

Get Init Values :<Get Init Values> button: Sets [Acquire] panel parameter to the default value.

Get FV Status: <Get FV Status> button: Acquires each setting value of FLUOVIEW software parameters and registers to PAPP window. The track which the parameters to be registered has to be highlighted.

Set to FV: <Set to FV> button: Sets PAPP parameters to FLUOVIEW software parameters.. The track which the parameters to be registered has to be highlighted.

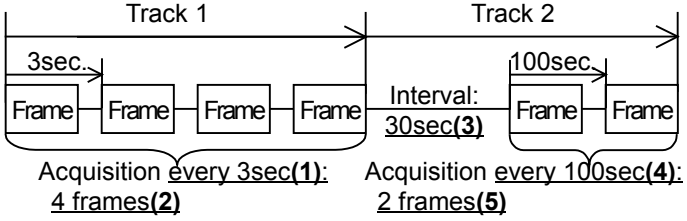
<Get FV Status> and <Set to FV> button can not be pressed during PAPP protocol is working.

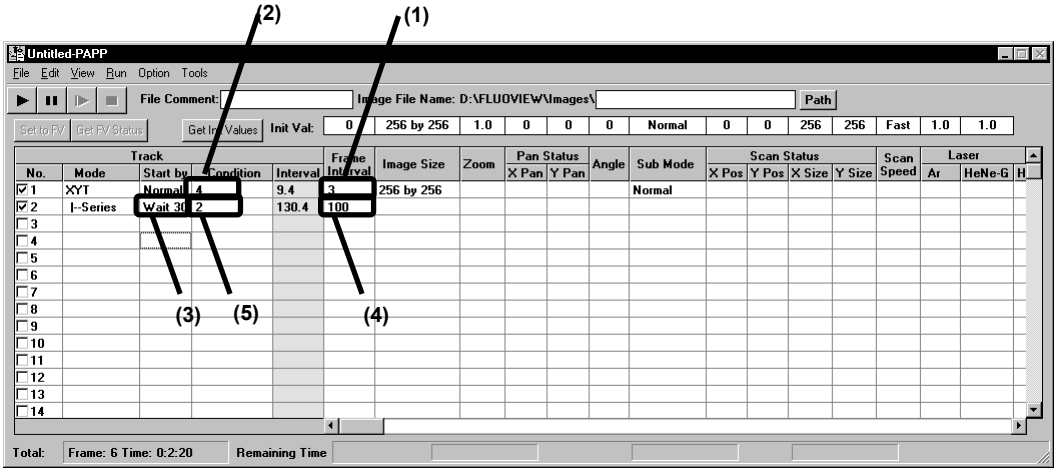
2-4-2 Editing the Protocol

The protocol of the Protocol Processor consists of several Tracks, and Track is a set of image acquisition condition and parameter settings.

The [PAPP] window is used to edit the protocol.

The Track needs to be edited as a set of acquisition condition, then deferent Track has to be created in case of the condition becomes different as shown below;





For details, see section 2-4-8," Example of assembling a protocol".

2-4-2-1 Description of Setting Items

The [PAPP] window has the setting items as described in the following.

<Get Init Values> button

Click to acquire the current settings in the [Acquire] panel as the initial values of the settings.

[Image File Name] text box

Enter the file name in which the image acquired will be saved.

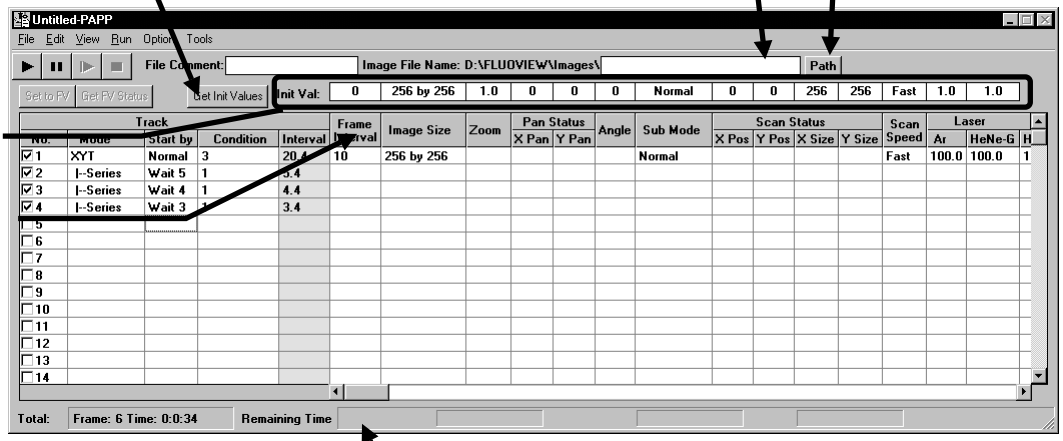
<Path> button

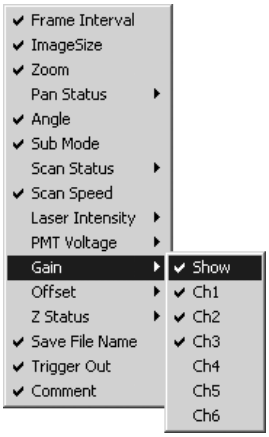
Specify the folder for saving the image acquired.

[Init Val] box

Shows the initial values of the settings.

When each item on each column is mouse-right clicked, pop up menu as shown below appears. It is possible to select Display/Not to display of each column.





[Remaining Time]

Shows the time till the end of protocol by means of figures and indicators. When [Alarm in Interval remaining time] in the [Option] menu is checked, beep tones can be generated at 30 seconds before the end of protocol (single beep), 20 seconds before it (two beeps), between 10 seconds and 5 seconds before it (a beep every second) and between 5 seconds before it and the actual end of protocol (successively).

·Setting items, particularly each laser of [Laser Intensity], [PMT Voltage], [Gain], [Offset] and [C.A] may be different, depending upon the system; and usable laser and channel on the system are displayed in each column of laser and channel.

·Value in each column is set in FLUOVIEW software right after protocol execution.

·When [Init] is selected in each column, the default value set in [Init Val] box is used.

·Blank cell refers to the setting of one previous track.

·Width of each row can be adjusted by mouse drag action on border line of row. By double click action over the border, the width can automatically be adjusted according to the length of characters.

Only available Laser type and PMT channel column is used with [Laser Intensity] and [PMT Voltage].

The values in each cell are applied to the [Acquire] panel at the beginning of the protocol.

When “Init” is specified in a cell, the value set in the [Init Val] box is applied.

The blank cell applies the value set to the track immediately above.

The width and height of PAPP window is adjustable. Width of each row is also adjustable.

The columns are used to set the following data.

No.: Track number. The Track is available if it is checked.

Mode: Select the mode of the track.

New mode : Assign primary image acquisition mode. The scan mode listed below can be assignable.

Series mode: This mode can set the previous scan mode to acquire consecutive XYT, XYZT, or XZT image with the acquisition parameter modified.

Sub-menu of New mode	Description	Series
XY	Acquires XY image.	X
XYT	Acquires XYT image.	O
XYZ	Acquires XYZ image.	X
XYZT	Acquires XYZT image.	O
XT	Acquires XT image.	X
XZ	Acquires XZ image.	X
XZT	Acquires XZT image.	O
Pt*T	Acquires Point-T image.	X

O Series mode (track for linkage) can be connected continuously.

X Series mode (track for linkage) cannot be connected continuously.

TIP

When [Mode] column is changed and initializations for all subsequent columns are required, put a check in [Refresh when changing Mode]. When information registered is not changed, uncheck [Refresh when changing Mode] at [Option] menu.

Command : The MACRO command mode.

For : Start of repetition (repetition processing using variables)

Next : End of repetition (repetition processing using variables)

Start by: Select the image acquisition start method.

Normal : Starts image acquisition when protocol comes the Track.

Trigger : Starts image acquisition after receiving external trigger input.

Wait : Starts image acquisition after wait time describes the track, the unit is second.

Condition: See the following table in case that [New mode] or [Series mode] is selected in [Mode].

When “Command” is selected in column [Mode], the macro command can be enter to the track. For the macro commands detail, see section 2-4-2-2, “Command List”.

When “For” is selected in column [Mode], enter the repetition condition using a variable. For the description of the repetition processing, see section 2-4-2-3 , “Protocol Repetition Processing”.

Sub-menu of New mode	**Condition column of New Mode**		**Condition column of the following Series Mode**
	Change	Input details	
XY	X	Set 1 automatically	
XYT	O	Set number of images to be acquired	Enter number of slices to acquire
XYZ	X	Set 1 automatically	
XYZT	O	Set number of images to be acquired	Enter number of slices to acquire
XT	O	Set number of lines	
XZ	X	Set 1 automatically	
XZT	O	Set number of lines	Enter number of lines to acquire
Pt*T	X	Set 1 automatically	

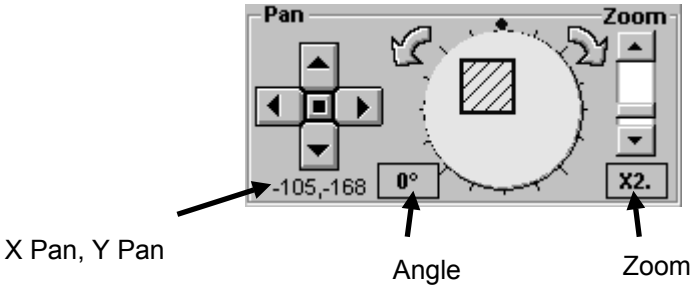
Interval: When “Frame” is selected in column [Mode], time required to execute the track is displayed. Change of [Interval] column cannot be done in case of [Series mode].

Frame Interval: Enter the interval time between image acquisitions in number of seconds. A number between 0.1 and 60000.0 can be entered.

Image Size: Select acquisition image pixels. Select either [256 by 256], [320 by 240[, [512 by 512], [640 by 640], [800 by 600], [1024 by 768], [1024 by 1024] or [2048 by 2048] at [X-size by Y-size] list box.

Zoom: Enter the zoom ratio in the range between 1.0 and 10.0.

Pan Status: Enter Pan value, refer to [Pan] [Zoom]group box in [Acquire] panel.



FLUOVIEW software [Pan][Zoom] Group Box

TIP

Pan value can not enter the following condition.

· Mode is set to any of XY, XYT, XYZ or XYZT.

· SubMode is Normal.

· Zoom is set to 1 (1.0 x).

TIP

Variables are available in [For] statement for X Pan and Y Pan.

In addition, four-rule calculation is possible.

For details about how to use variables, see One Point of 2-4-8 “2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)”.

Angle: Assign rotated image scan angle of field of view. 0 through 359 can be entered. Angle can not set at a certain sub scan mode, and a certain hardware. Please see 2-4-10 [Restrictions at [PAPP] window input time]. for entered angle value restriction.

TIP

Variables are available in [For] statement for Angle setting.

In addition, four-rule calculation is possible.

For details about how to use variables, see One Point of 2-4-8 “2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)”.

Sub Mode: Sub Mode is for scan mode setting of New mode appears by click on Mode area. A certain scan mode may not set because of hardware configuration. See 2-4-10 “Restrictions at [PAPP] window input time” for restriction of scan mode setting.

In this case of Normal scan, there might be scanned before the scan mode changes.

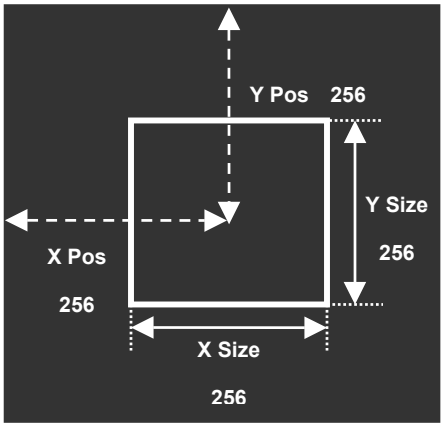
Scan Status: Change scanning position and its size with the selected scan mode. See 2-4-10 “Restrictions at [PAPP] window input time” for restriction of Scan status input.

TIP

Variables are available in [For] statement for X Pos, Y Pos, X Size, Y Sieze. In addition, four rule calculation is possible.

For details about how to use variables, see One Point of 2-4-8 “2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)”.

(0, 0) (511, 0)



Examples of parameters: Default clip scan position setting which appears when the clip scan is selected with 512 x 512 pixel.

· X Pos : 256
· Y Pos : 256
· X Size : 256
· Y Size : 256

(0, 511) (511, 511)

Scan speed: Sets image acquisition speed from “Fast”, “Middle”, “Slow” and “Init” in the pull-down menu.

The above speed is the same with those of [Scan Speed] group box in [Acquire] panel. In case that [Fast] or [Fast Clip] is selected in [Sub mode], [Scan Speed] is automatically set to [High Speed].

Laser Intensity: Enter the intensity setting for each laser. The setting is 0 to 100, and the unit is %. When it sets to 0, a laser shutter is closed.

REX Mask: Select the REX mask file from the pull-down list whether to use the REX mask which is displayed on FLUOVIEW software or to disable the mask by selecting “None”.

[REX Mask] appears only when AOTF (FV5-COMBA) is equipped.

PMT Voltage: Enter the PMT voltage of each channel. The setting is 1 to 1100, and the unit is V.

Gain: Enter the gain of each channel. The setting is 1.0 to 10.0.

Offset: Enter the offset of each channel. The setting is 0 to 100, the unit is %.

C.A.: Enter the confocal aperture diameter for each channel. The setting is 50 to 800, the unit is micron.

XY Stage Track No: When multi-point time lapse software is used, track number of multi-point time lapse software that meets to this track will be displayed. When multi-point time lapse software is not used, the display does not appear.

Zstatus: Enter Z stage information.

Start Pos: Enter the Z-scanning start position of cross section of which observation is required.

Stop Pos: Enter the Z-scanning stop position of cross section of which observation is required.

Step Size: Enter the number of steps in Z direction.

Slices: Enter the number of image slices to be acquired

Save FileName: Input file name for acquired image. Image would be saved under path of [Image File Name:].

One Point!

It is possible to designate folder in [Save File Name] column. For example, if [R001 \Image001] is entered, folder – [R001] is created under path of [Image File Name:] and image file is saved under the name of [Image001].

Trigger Out: Select the trigger output from “00”, “01”, “10” and “11” in the pull-down menu.
Comment: Enter any comment as desired.

In case of the range of acceptable values are specified for a cell, it is checked according to the specified range. If a value out of the specified range is entered, it is corrected in the vicinity value.

TIP

Variables will be created as defined with For statement for input value. Also, four operations are possible. Regarding the use of variables, see [One Point] in 2-4-8 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing).

2-4-2-2 Command List

The list of macro commands is shown below.

When “Command” is selected in column [Mode] in the [PAPP] window, enter the macro command to be carried out for the track. The macro command to be entered can be selected from the drop-down list. And enter each value directly using keyboard.

Command	Entry form	Description
Append	Append␣ [FloderName]␣ [,AppendFileName] ␣[,T/Z/AN] ␣[,Date/Name]	It appends images that exist in the same folder into one file. [FolderName]: Specify the folder that holds a file to be appended. The default is [C:FLUOVIEW¥Images]. [AppendFileName]: Specify the file name which use after append. The default is [Append_Year/Month/Day]. [T/Z/AN]: Specify the appended image data type from T series image, Z series image or animation image. The default is T series. [Date/Name]: Specify the order of append, date or name. The default is date.
CopyROI	CopyROI	Copies active ROI of FLUOVIEW image into memory in the track is in use.
Dye	Dye␣string1 [,string2][,string3] [,string4]	It selects dye methods that use String 1 to 4. 4 kinds – 1 to 4 - can be set. Dye colors that can be selected are of the ones displayed in dyeing method when [Dyes] sub panel of [Acquire] panel on FV300/500/1000 is opened. Fluorescent reagents are set in sequence of reagent set in [string]. <E.g> In case that FITC and Cy5 are selected as dye method, enter [Dye FITC, Cy5].
EnCh	EnCh␣ Ch , [ON/OFF]	It sets Valid/Invalid of each channel. In Ch, channel number is designated. Depending upon system configuration, max. 5 channels can be selected. When the channel

Command	Entry form	Description
		designated is to be Valid, select ON and, in case that Invalid is to be set, select OFF. <E.g> In case that channel 1 is to be Valid, enter [EnCh1, ON].
Filter	Filter␣[NORMAL /KALMAN/PEAK] [,value]	[NORMAL/KALMAN/PEAK]: It defines type of integration. NORMAL: No integration KALMAN: KALMAN filter PEAK: Peak detecting integration value: In case that KALMAN is selected, integration cycle of KALMAN filter is set and, in case of PEAK, max. addition cycle at PEAK integration is set. In case that NORMAL is selected, no setting is required. <E.g> In case that integration is required 4 times, using KALMAN filter, enter [Filter KALMAN, 4].
Gain	Gain␣ch,␣value	Sets the Gain value of channel. <E.g.> Enter “Gain 2, 50” to set 50 to Gain of channel 2.
LivePlot	LivePlot␣ [ON/OFF]	[LivePlot ON] is selected, LivePlot is displayed. In case of [LivePlot OFF], LivePlot is not displayed. (This is TIEMPO option.)
Merge	Merge␣ MergeFile1, MergeFile2 [,Ch(s)][,Ch(s)]	Merges image data that is located under FLUOVIEW software. The following example is to merge 2ch of File1 and 3ch of File2. <E.g> [Merge C:¥FLUOVIEW¥Images ¥File1,C:¥FLUOVIEW¥Images¥File2,2,3]
Message	Message␣message	Displays a message to suspend executing a

Command	Entry form	Description
		protocol.
Offset	Offset␣ch␣value	Sets the Offset value of channel. <E.g.> Enter “Offset 2, 5” to set 5 to the offset value of channel 2.
PasteROI	PasteROI	Pastes ROI to the next track. Note: Before the paste, Normal scan is executed one time.
Pause	Pause	Suspends executing a protocol.
ReceiveCOM	ReceiveCOM␣ message	Receives RS-232C command from other PC. The following data returns after command is executed. Completes normally: “OK” + Received characters Ends with error: “NG” + Received characters. For further details, see 2-4-7.
Save	Save	Saves whole series of images.
SendCOM	SendCOM␣ message␣[,0/1]	Sends data through RS-232C to other PC. The following data has to be returned in synchronous mode,. Completed normally: “OK” + sent characters Ends with error: “NG” + sent characters For further details, see 2-4-7.
Size	Size␣X, ␣Y	Sets the image size in pixel. <E.g.> Enter “Size 512, 512” to set 512X512 to the image size. Note: The images acquired before and after executing the Size command cannot be saved together. In the previous or succeeding track, execute the Save, Store, or Stop&Save command to save the images acquired. Note) Image size can be set at the following

Command	Entry form	Description
		combination. Be careful that other combination than the following cannot be entered. (256, 256) (320, 240) (512, 512) (640, 480) (800, 600) (1024, 768) (1024, 1024) (2048, 2048).
Stop&Save	Stop&Save	Saves all the images acquired before the Stop&Save command is executed. Note: The images are saved separately from the images acquired in succeeding tracks.
Store	Store	Opens a new image window to display the image.
Zlock	Zlock␣[ON/OFF]	It sets Valid/Invalid of Z motor lock. Note that fine tuning handle and coarse tuning handle cannot be manually operated when Z motor is locked. In case that manual operation is required by releasing Z motor lock, difference of Z stage position displayed on [Zstage] sub panel of [Acquire] panel of FV300/500/1000 may occur. Use the handle after confirming the moving range adequately. In case that Z stage is controlled with use of command, lock Z motor. Do not turn fine tuning handle of microscope in the state that Zlock command is turned ON as it may cause failure. <E.g> In case that Z motor lock is required, enter [Zlock ON].
Zoom	Zoom␣value	Sets the zoom ratio. <E.g.> Enter “Zoom 5” to set 5 to the zoom ratio.

NOTE

The images acquired in different sizes cannot be saved together. Execute the Stop&Save command to save the image acquired before changing the image size.

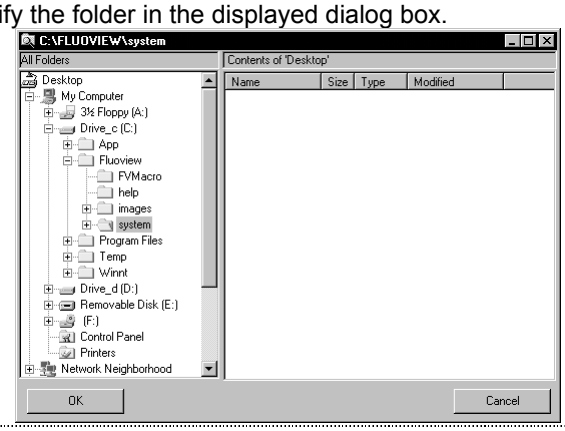
One Point!

Multiple image files saved using the Stop&Save command can be combined into a single time-lapse image file.

Combine the file by referring to section 2-6-6-1, "Appending two images"

TIP

The destination of file save using the Save and Stop&Save commands is the path displayed on the left of the [Image File Name] text box. If it is required to change the file save destination, click the <Path> button on the right of the text box and specify the folder in the displayed dialog box.



2-4-2-3 Protocol Repetition Processing

A protocol can be executed repeatedly for the specified number of times using a variable.

Use the For and Next commands to execute repetition.

The track enclosed between For and Next can be repeated for the number of times specified using a variable.

Create the For track immediately before and the Next track immediately after the track to be repeated by specifying a variable. After entering [For] in column [Mode], enter the variable name and repetition condition in column [Condition].

Command	Condition	Description
For	Variables␣=␣start␣To␣end␣(Step␣n)	Define a variable name in <Variables>, the start value in <start>, end value in <End> and an integer representing the number of steps in <n>. The range to which the variable is specified will be executed repeatedly. The number of steps can be entered as required. If it is not entered, the default repetition count of 1 will be applied. <Ex.> To repeat processing from the next track to [For] to the previous track to [Next] until variable i becomes from 1 to 5 (5 times), enter ”i = 1 To 5”.
Next	None (It is not required to enter [Condition].)	Specify the turnover point of repetition. The processing will be executed until the track immediately before [Next], returns to the track immediately after [For] and repeats from there.

Processing is possible only when [For] and [Next] are entered as a set. Their combination allows nesting for up to 16 times.

One Point!

It is possible to use variables in [For] statement in other cell of protocol processor.

<E.g>

It is possible to change laser intensity value every image acquiring time. When laser intensity of argon laser is defined as variable N, it creates protocols that acquire image with interval of step 10 by N equal to 10 to 100.

Track					Scan Speed	Laser Intensity		
No.	Mode	Start by	Condition	Interval		Ar	HeNe-G	HeNe-R
☑ 1	For	Normal	N = 10 To 100					
☑ 2	XY	Normal	1	0.4		N		
☑ 3	Next	Normal						

1. Enter [N = 10 To 100 Step 10] for Condition column in No.1 track.
2. Define laser intensity of argon laser as variable N for No.2 track.
3. Define return of repetition for No.3 track.
4. Select <Start> button.

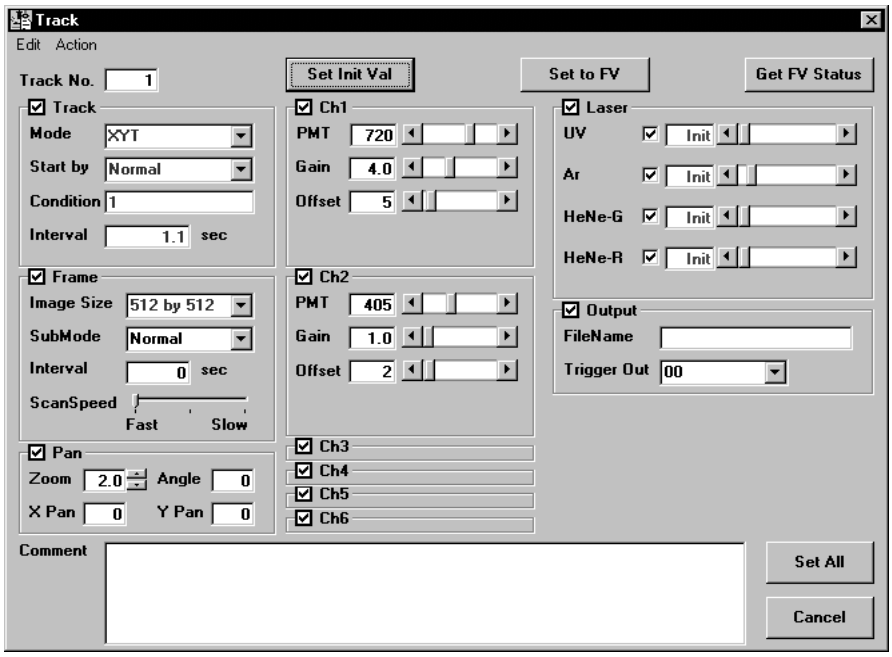
   Image will be acquired by changing laser intensity of argon laser by 10% as shown below.

	1st	2nd	3rd	4th	5th	…	10th
Laser (%)	10	20	30	40	50	…	100

2-4-2-4 Input supporting function

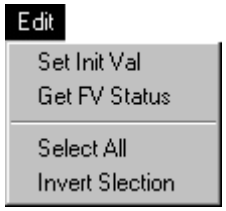
[Track] window is available as input supporting function for each track.

[Track] window appears by double-clicking No of [PAPP] window.



- All of parameters that can be set for the Track can modify.
- Parameter that is deferent with the previous Track is colored with Red.
- Parameters that is modified on [Track] windows is colored with purple.
- When Set Init Val button is clicked, the setting of item checked at check box of group box name is changed to the value in [Init Val] of [PAPP] window and [init] is displayed.
- When Get FV Status button is clicked, the setting of item checked at check box of group box name is acquired from FLUOVIEW software.
- When Set to FV button is clicked, the setting of item checked at check box of group box name is set to FLUOVIEW software.
- Set Init Val <Set Init Val> button loads [Init Val] box in [PAPP] window into [Track] parameters.

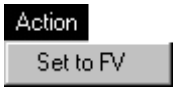
One Point!



It is possible to select [Set Init Val] and [Get FV Status] from [Edit] menu.

When [Select All] is selected from [Edit] menu, checks will enter all check boxes of group boxes.

When [Invert Selection] is selected from [Edit] menu, state of checks in all check boxes of group boxes will be inverted. When check is entered, the check will be removed and, when check is removed, the check is entered.



It is possible to select [Set to FV] from [Action] menu.

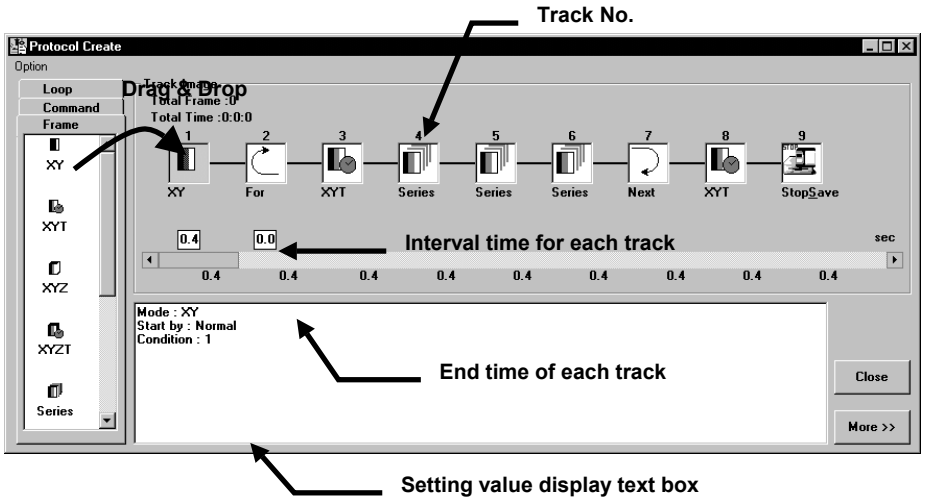
- Get FV Status <Get FV Status> button loads parameters which FLUOVIEW software has.
- By pressing Set All <Set> button, [Track] window setting is registered to [PAPP] window.
- Cancel <Cancel> button is for close [Track] window.

NOTE **Parameter changes in [Track] does not affect to FLUOVIEW software setting.**

NOTE **[Track] parameter can not modify nor register during the Track is running.**

2-4-2-5 Protocol procedure supporting function

[Protocol Creator] window helps to make procedure, especially time-series, easily..



When [Protocol Creator] that exists in [Tool] menu bar of [PAPP] window is selected, [Protocol Create] window appears. [Protocol Create] window is displayed at the position and in width when the window was previously closed.

- Left side of [Protocol Creator] window has [Frame] tab, [Command] tab and [Loop] tab.
  Track can be made by drag and drop those icon(s) into [Track Image].
  [Track] window appears and setting value can be entered.
- [Track] window does not appear with every drag and drop if [Show detail (when the drop)] in [Option] menu of [Protocol Creator] is not checked.
- A pair of [For] and [Next] icon is added after drag and dropped [Loop] icon in [Loop] panel into [Track Image]. [For] and [Next] icon can move to the Track by dragging the appropriate position.
- [Track] window opens by double-clicking icon on [Track Image] group for detail setting.
- Details of parameters are displayed in setting value display text box by clicking icon.
- Interval time of each Track can be entered into the text box.
- The parameters affect to the [PAPP] window by pressing Close <Close> button.

TIP

By pressing <More> button, the detail information is displayed. By pressing <Less> button, close the information display..

NOTE

During [Protocol Creator] window is displayed, [PAPP] window can not be operated.

2-4-3 Saving the Protocol

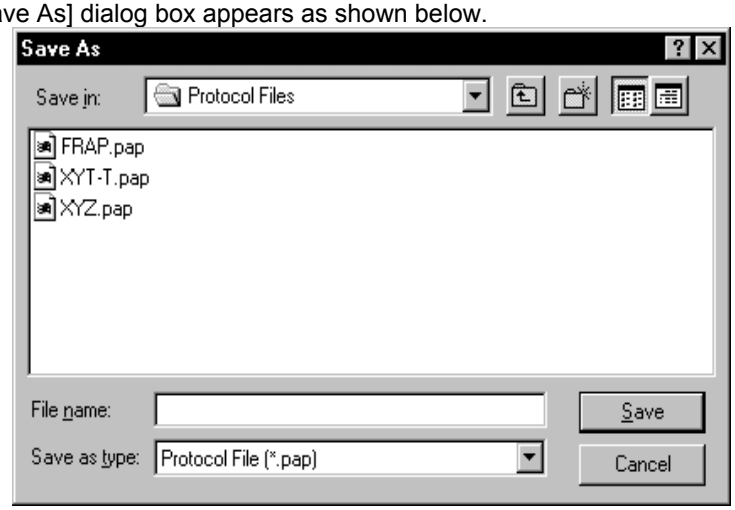
The Protocol Processor creates and saves protocol files in the CSV file format.

TIP

The CSV file format applies the text format delimiting the items with comma in a database. Protocol files can be saved only in the CSV format.

1. In the [File] menu, select [Save] or [Save As].

   The [Save As] dialog box appears as shown below.



2. To change the save destination drive and/or directory, use the [Save in:] drop-down list.

3. Enter the file name in the [File Name:] text box.

4. Select file type, .pap or .csv, with [Save as type:] dropdown list.

5. Click the <Save> button.

NOTE

If a CSV file having the same file name as the file name entered above already exists, a dialog box appears to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <No> button and use another file name for saving.

2-4-4 Executing the Protocol

The created protocol can be executed to acquire time series images.

<Start> button

1. In the [PAPP] window, click the <Start> button.
   The number of rows in the [No.] list in the [PAPP] window becomes 2, and the window display is reduced. The row being executed is show in reverse display and the reverse-displayed row changes in synchronism with the change in the executed track.

One Point!

The [PAPP] dialog box can be arranged as follows;

- **Always display the [PAPP] dialog box at the front.**
  Check “On Top” in the [View] menu in the [PAPP] dialog box.
- **Display the [PAPP] dialog box in full screen.**
  Check “Full Screen” in the [View] menu in the [PAPP] dialog box.
- **Shrink the [PAPP] dialog box to the bottom of the screen while executing a protocol.**
  Before executing a protocol, check the “To Bottom on Running” in the [View] menu in the [PAPP] dialog box.
- **Shrink the [PAPP] dialog box at any position in the screen while executing a protocol.**
  Before executing a protocol, check “Shrink on Running” in the [View] menu in the [PAPP] dialog box and move the [PAPP] dialog box to anywhere in the screen.
- **[PAPP] window and image window are separated and displayed.**
  Prior to executing protocol, select “Share the screen with FV” from [View] menu of [PAPP] window and make it checked state. When it is done so, “To Bottom on Running” and “Shrink on Running” are simultaneously checked. Further, when check of either “To Bottom on Running” or “Shrink on Running” is removed, the check of “Share the screen with FV” is also removed.

<Break> button

2. When stopping the protocol, click the <Break> button.
   When the protocol is executed again, click the <Start> button.

TIP

When clicked while the protocol is executed, the <Break> button performs the same function as the <Stop Scan> button in the [Acquire] panel.

<End> button

3. To stop the protocol without executing it till the end, click the <End> button.

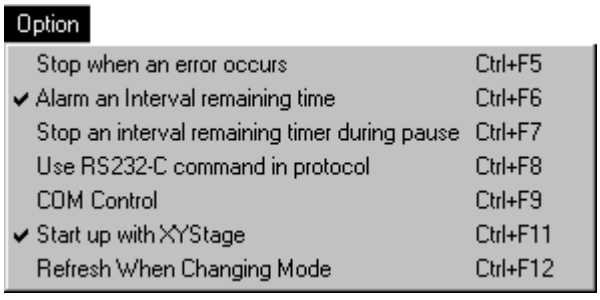
TIP When clicked while the protocol is executed, the <End> button performs the same function as the <Series Done> button in the [Acquire] panel.

One Point!

The protocol can be processed using the items under the [Run] menu in the [PAPP] window.

- **Start executing the protocol.**
  Select “Start” in the [Run] menu in the [PAPP] window.
- **Suspend executing the protocol.**
  Select “Break” in the [Run] menu in the [PAPP] window.
- **Fast forward the protocol.**
  Select “Skip” in the [Run] menu in the [PAPP] window.
- **Stop executing the protocol.**
  Select “End” in the [Run] menu in the [PAPP] window.

One Point!


Option
Stop when an error occurs Ctrl+F5
Alarm an Interval remaining time Ctrl+F6
Stop an interval remaining timer during pause Ctrl+F7
Use RS232-C command in protocol Ctrl+F8
COM Control Ctrl+F9
Start up with XYStage Ctrl+F11
Refresh When Changing Mode Ctrl+F12

- When [Stop when error occurs] is selected – checked from [Open] menu, process is stopped in case that error occurs during protocol processing at the track where the error occurred. In case that the check is removed, the process goes to next track when error occurred.
- When [Stop an interval remaining timer during pause] is selected – checked from [Option] menu, the time at pause is not counted. In case that the check is removed, the time at pause is counted and, if the time counted exceeds the time set for track, the process will be immediately started at next track when protocol is executed again.

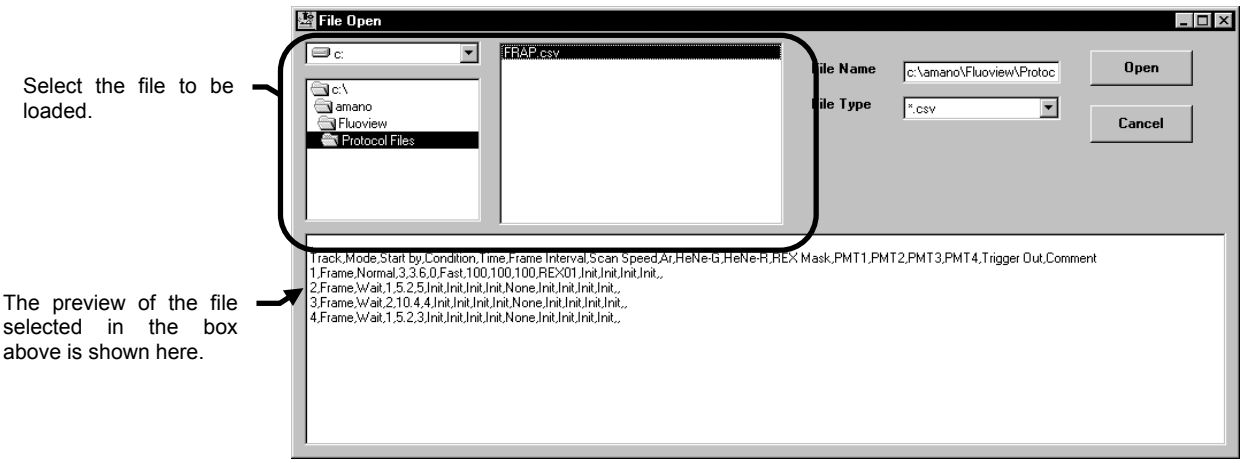
2-4-5 Loading a Protocol

The Protocol Processor creates and saves protocol files in the CSV file format.

The following procedure loads a CSV file (extension .pap or .csv) for editing the protocol in it.

1. Start the Protocol Processor software and display the [PAPP] window.
   For details, see section 2-4-1, “Starting the Protocol Processor”.

2. In the [File] menu, select [Open].
   The [File Open] dialog box appears as shown below.



3. Specify the file to be loaded in the box at the top left of the dialog box.

4. Click the <Open> button.
   The loaded track data will be displayed in the [PAPP] window.

2-4-6 Loading a protocol of previous format

FLUOVIEW software Ver4.2 inform the protocol data conversion when the software reads previous version of the data.

The rule of the conversion is explained below.

Rule A

- Before Ver4.2, Frame = XYT or XYZT is converted to XYT or XYZT in count of Z slice parameter.
  - If Z Slices = 1, the Frame becomes XYT.
  - If Z Slices is more than 1, the Frame becomes XYZT.
  - If the Frame is continuous one, Frame becomes Series.

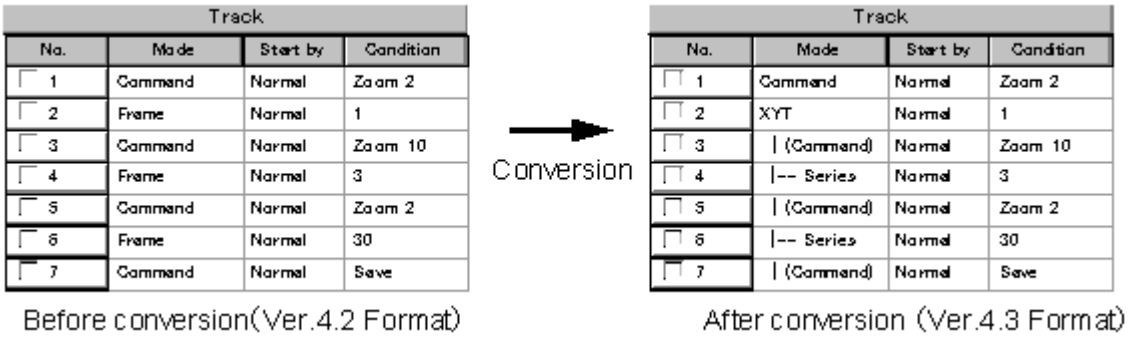

Track
No. Mode Start by Condition
1 Command Normal Zoom 2
2 Frame Normal 1
3 Command Normal Zoom 10
4 Frame Normal 3
5 Command Normal Zoom 2
6 Frame Normal 30
7 Command Normal Save
Conversion
Track
No. Mode Start by Condition
1 Command Normal Zoom 2
2 XYT Normal 1
3 | (Command) Normal Zoom 10
4 |-- Series Normal 3
5 | (Command) Normal Zoom 2
6 |-- Series Normal 30
7 | (Command) Normal Save
Before conversion(Ver.4.2 Format)
After conversion (Ver.4.3 Format)

Fig. 2-50 Sample Program (FRAP)

Rule B

- Before Ver4.2, Frame (Separate) is converted with condition of Track and Z slices.
- If Condition = 1, and Z slice =1, Frame becomes XY.
- If Condition is more than 1, and Z slice = 1, Frame becomes XYT.
- If Condition = 1, and Z slice is more than 1, Frame becomes XYZ.
- If Condition and Z slice are more than 1, Frame becomes XYZT.

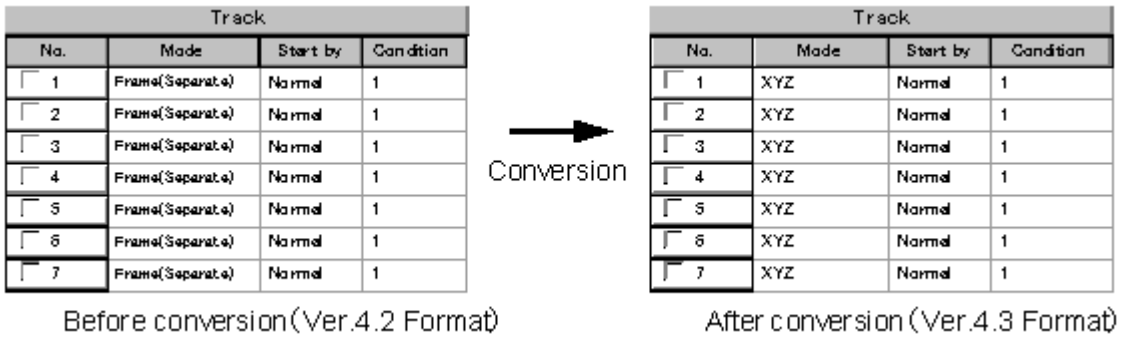


Fig. 2-51 Multi-Point Time Lapse Protocol

Rule C

- For and Command in Series appears with indented + ( ).
- Combined For – Next structure becomes single layer.

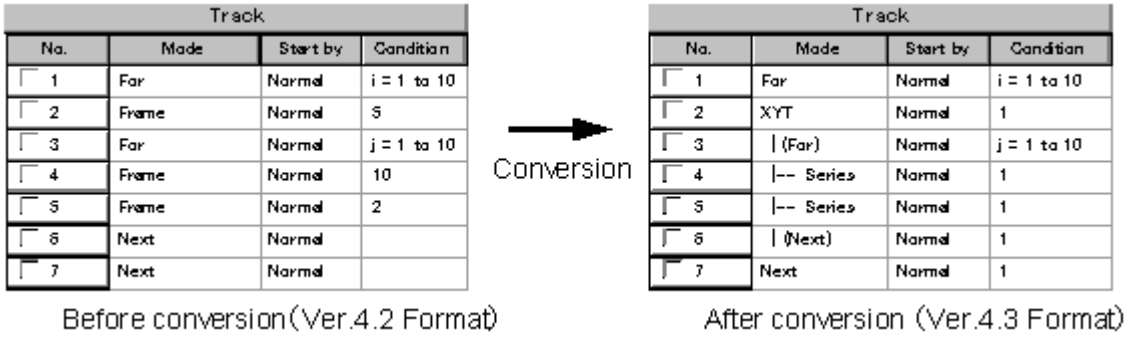

Track
No. Mode Start by Condition
1 For Normal i = 1 to 10
2 Frame Normal 5
3 For Normal j = 1 to 10
4 Frame Normal 10
5 Frame Normal 2
6 Next Normal
7 Next Normal
Conversion
Track
No. Mode Start by Condition
1 For Normal i = 1 to 10
2 XYT Normal 1
3 | (For) Normal j = 1 to 10
4 |-- Series Normal 1
5 |-- Series Normal 1
6 | (Next) Normal 1
7 Next Normal 1
Before conversion (Ver.4.2 Format)
After conversion (Ver.4.3 Format)

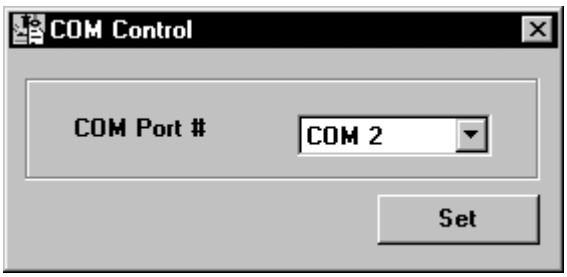
Fig. 2-52 Protocol Using [For] Statement

2-4-7 COM Communication function

PAPP can communicate with RS-232C to the other PC. The data for sending and receiving should described with Macro commands. For detail of Macro command, see 2-4-2 [2 Table of commands].

Select COM port of PC.

- Click [Option] menu in [PAPP] window, then click [COM Control].
  [COM Control] dialog appears.
- Select COM port # from [COM Port#] dropdown list.
- By clicking <Set> button to be able to use the port.



[COM Control] dialog box

- "SendCOM" and "ReceiveCOM" commands are available when [Use RS232-C command in protocol] in [Option] menu is checked.
- In case of using "SendCOM" command with synchronous mode, the following data should be return from the other PC.

  When the command Completes normally
  'OK' + Transmitted command
  When the command ends with error
  'NG + Transmitted command

- In case of receiving "ReceiveCOM" command, the following data will be returned.

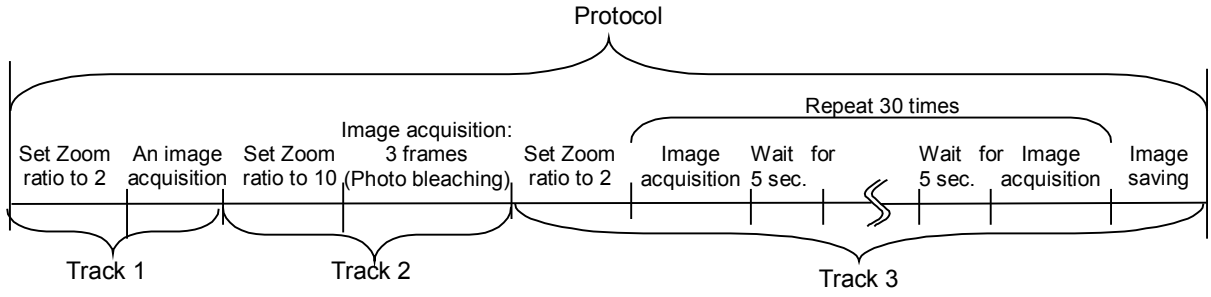
  When the command completed normally
  'OK' + Received character strings
  When the command ends with error
  'NG' + Received character strings

2-4-8 Example of assembling a protocol

This section describes an example of assembling a protocol.

2-4-8-1 Example of Setting Procedure of FRAP Observation

An example of editing a protocol is described below assuming the FRAP observation.



Initial image acquisition: Track 1 sets the zoom ratio to 2 x, and acquires an image.

Photobleaching: Track 2 sets the zoom ratio to 10 x, and acquires image 3 times to make photobleach on the specimen.

Recovery measurement: Track 3 sets the zoom ratio to 2 x, and acquire image 3 times with 5 seconds interval to observe the recovery. Then, stores the image.

TIP

Execute a XY repeated scanning and adjust the set values for image acquisition before executing the protocol.

1 Setting the observation condition

Before setting and starting the FRAP observation using the Protocol Processor, execute a repeated scan and set the condition for the observation.

Assuming FITC to Channel 2 and image size to 320X240 pixels, the following figure shows an example of image acquisition.

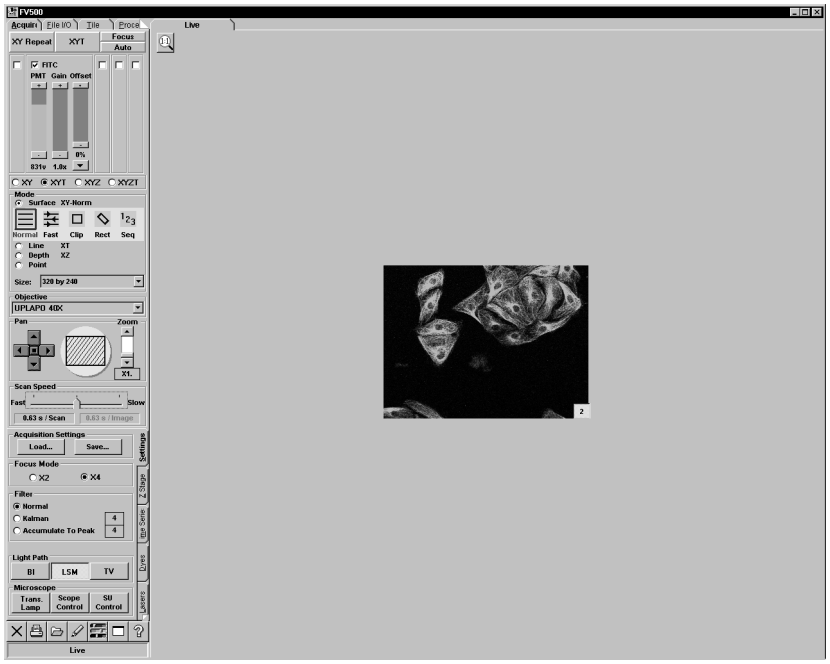


Fig. 2-53 Panel After a Repeated Scan

2 Displaying the Real-Time Graph (TIEMPO option software required)

1. Specify the region in the image to display the real-time graph.
   For specifying method, refer to section 2-4-3, “Specifying the Regions Where Intensity Graph is Displayed in Real Time”.
2. Display the [Tiempo] sub-panel in the [Acquire] panel to check the [Show live plot] check box.
   For checking method, refer to section 2-4-4, “Displaying the Real-Time Graph”.

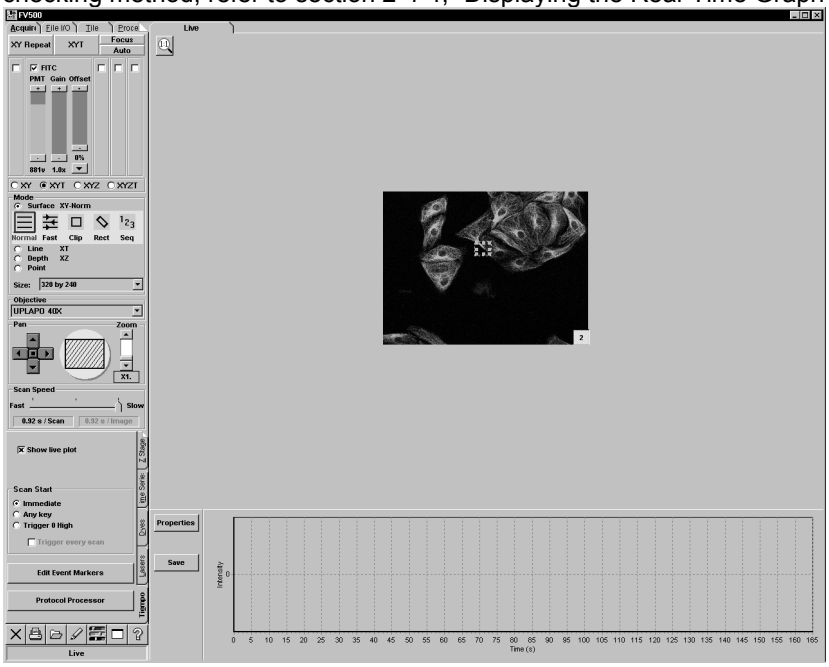


Fig. 2-54 Panel Displaying the Real-time Graph

3 Editing the Protocol

Start and edit the protocol after setting of the observation condition has been completed.

For starting method, refer to section 2-4-1, “Starting the Protocol Processor”.

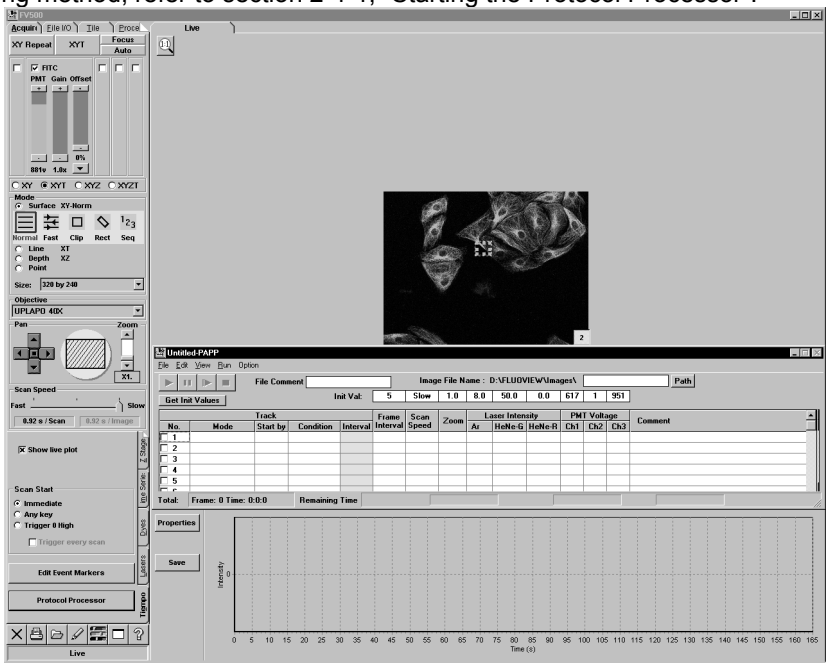


Fig. 2-55 Panel Displaying the [PAPP] window

Set the [PAPP] window as shown below;

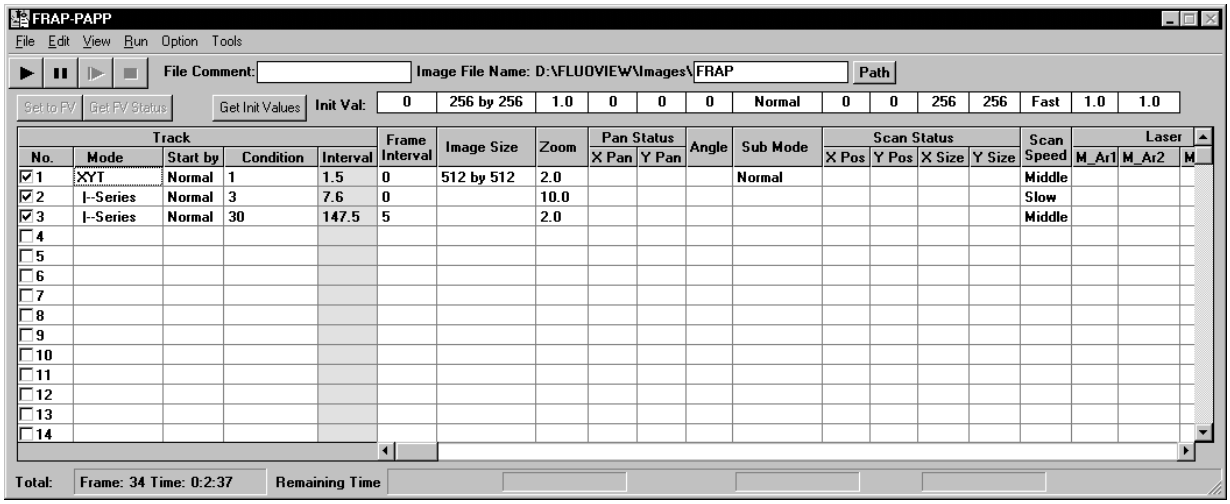


Fig. 2-56 [PAPP] window Setting an Example of Observation Condition

Set each Track as sown below;

- **Setting Track 1**

  In XYT observation, set the zoom ratio to 2X and acquire an image before bleaching.

  1. Select [New mode]-“XYT” in [Mode] of [Track].
  2. Select “Normal” in [Start by] of [Track].
  3. Enter “1” in [Condition] of [Track].
  4. Enter “0” to [Frame Interval].
  5. Enter “2” to [Zoom].
  6. Select “Middle” in [Scan Speed].
  7. Enter the laser intensity value for image acquisition to [Laser Intensity]
  8. Enter the PMT value of each channel to [PMT Voltage].
  9. Enter a comment in [Comment] if necessary.

- **Setting Track2**

  In XYT observation, set the zoom ratio to 10X and acquire three images. (Irradiate strong laser onto the region to bleach.)

  1. Select “Series mode” in [Mode] of [Track].
  2. Select “Normal” in [Start by] of [Track].
  3. Enter “3” to [Condition] of [Track].
  4. Enter “0” to [Frame Interval].
  5. Enter “10” to [Zoom].
  6. Select “Slow” in [Scan Speed] for fast photo-bleaching.
  7. Enter the laser intensity value for image acquisition to [Laser Intensity]
  8. Enter the PMT value of each channel to [PMT Voltage].
  9. Enter a comment in [Comment] if necessary

<Skip> button

TIP

To properly irradiate the laser for bleaching, enter a larger value in [Condition] and fast forward the tracks using the <Skip> button in accordance with the grade of bleaching.

- **Setting Track 3**

  In XYT observation, return the zoom ratio to 2X and acquire 30 images every 5 seconds. (Observe the change in fluorescence image with time after bleaching.)

  1. Select “Series mode” in [Mode] of [Track].
  2. Select “Normal” in [Start by] of [Track].
  3. Enter “30” to [Condition] of [Track].
  4. Enter “5” to [Frame Interval].
  5. Enter “2” to [Zoom].
  6. Select “Middle” in [Scan Speed]. (Return to the setting before bleaching.)
  7. Enter the laser intensity value for image acquisition to [Laser Intensity].
  8. Enter the PMT value of each channel to [PMT Voltage].
  9. Enter the image file name to be stored to [Save FileName].
  10. Enter a comment in [Comment] if necessary.

One Point!

The protocol to perform the following operations can be assembled after setting the observation conditions.

- **Specifying the region for bleaching in the laser irradiation mode using REX mask files.**

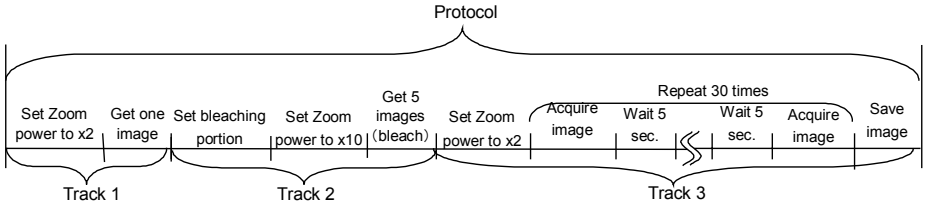
In Track 4, select a REX mask file in [Mask] of [REX]. Then in the laser irradiation mode specified by the REX mask file selected, images can be acquired. (When using the system with AOTF.) To select a REX mask file, display the REX mask file in the image window in advance.

<The setting example>

Track					Frame Interval	Image Size	Zoom	Sub Mode	Scan Speed	Laser Intensity			REX Mask	Save FileName	Trigger Out
No.	Mode	Start by	Condition	Interval						Ar	HeNe-G	HeNe-R			
☑1	XYT	Normal	1	10.0		512 by 512	2.0	Normal	Middle						
☑2	I-Series	Normal	3	30.1			10.0		Slow	100.0	0.0		REX_Live		
☑3	I-Series	Normal	30	300.7			2.0		Middle	INIT	INIT		None	BLEACH	
☐4															

For details, see section 2-2-11, “Image Acquisition in the Laser Excitation Mode”.

- **Setting bleaching region by use of rectangular image acquisition (RECT).**



Reference image acquisition:

Acquire an image with the Zoom power 2 x in Track 1 in XYT observation.

Photo-bleaching:

Expose laser to photo-bleach with 5 images of acquisition using ZoomIn mode in Track 2 in XYT observation.

Recovery observation:

Acquire 3 images in XYT observation every 5 seconds with the zoom power 2 x, the field of view is the same with the reference image, and the images are stored in Track 3.

<Example>

Track					Frame Interval	Image Size	Zoom	Pan Status		Angle	Sub Mode	Scan Status				Scan Speed
No.	Mode	Start by	Condition	Interval				X Pan	Y Pan			X Pos	Y Pos	X Size	Y Size	
☑1	XY	Normal	1	1.0	0	512 by 512					Normal					
☑2	XYT	Normal	5	1.6	0	512 by 512	2.0	0	0	0	ZoomIn	44	128	256	256	Fast
☑3	XYT	Normal	30	146.5	5	512 by 512					Normal					
☐4																

4 Saving the Protocol

Save the edited protocol data.

The protocol can also be saved after being executed.

1. Select [Save] or [Save as] in the [File] menu in the [PAPP] window.

   Save using the dialog box displayed.

   For details, see section 2-4-3, “Saving the Protocol“.

5 Executing the Protocol

Executes the protocol.

<Start> button

1. Click the <Start> button in the [PAPP] window.

   For details, see section 2-4-4, “Executing the Protocol”.

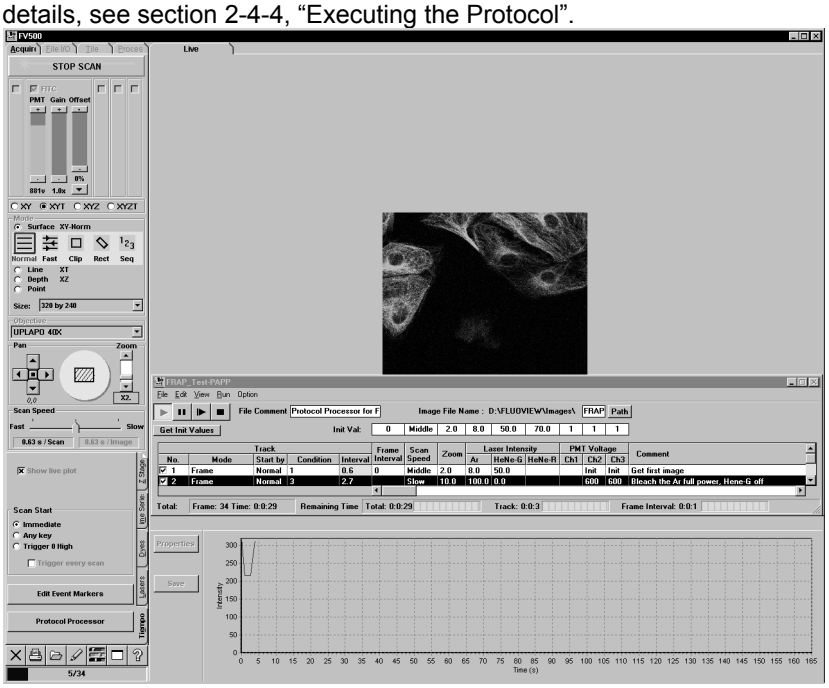


Fig. 2-57 Panel While Executing the Protocol

6 Exiting the Protocol

Terminate the protocol after acquiring the images.

The images saved.

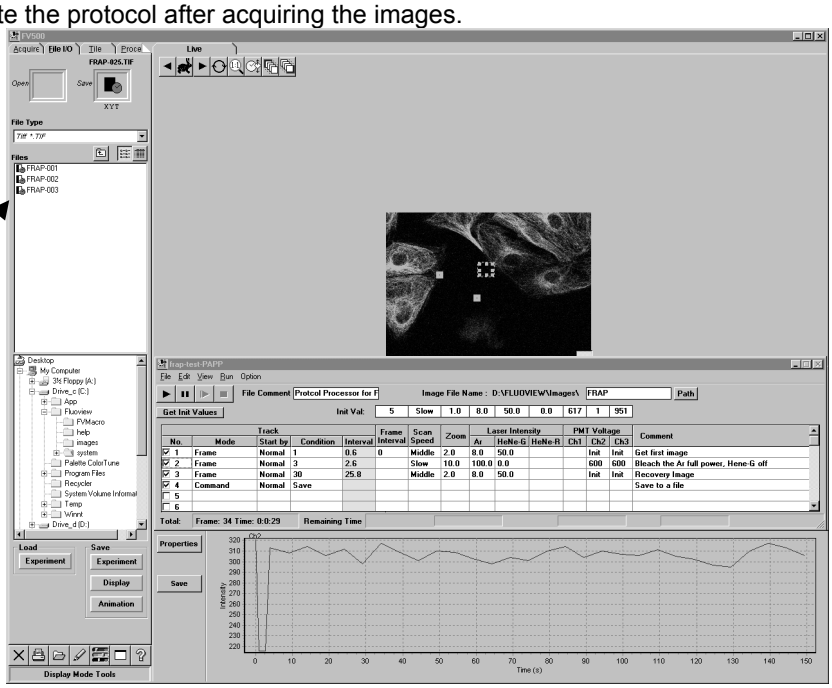
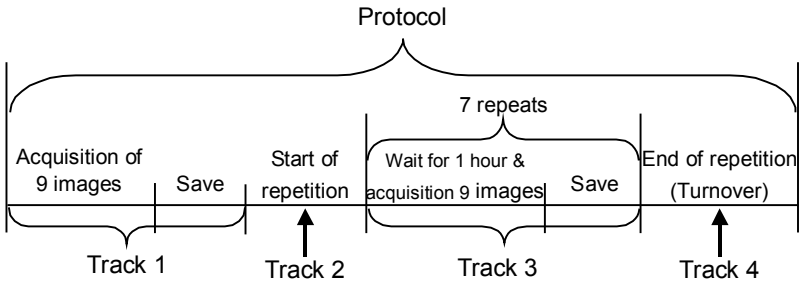


Fig. 2-58 Panel When Exiting the Protocol

2-4-8-2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)

An example of editing a protocol using the repetition processing is described below assuming the XYT observation including the GFP recovery observation for hours.



Reference image acquisition: Acquire 9 images with the zoom power 2 x, then save the images acquired in Track 1.

Repeated Protocol start : Repeats protocol of Track 3, the repeated times is 7 which is assigned with For statement in Track 2.

Long interval image acquisition: Acquire 9 images every one hour, and the image is stored in Track 3.

Repeated Protocol end: Track 4 defines whether ends or continues Track 3 protocol.

TIP

Specify the Z range and select XYZT mode before starting the XYZT observation. For details, see section 2-2-1-4, “Setting the Observation Condition”.

One Point!

The variable in the [For] statement can also be used by another cell in the protocol processor.

<Setting example>

It is also possible to change the laser intensity value at every image acquisition. In this case, assume that the laser intensity of the Ar laser is variable N and create a protocol for acquiring image as N varies from 10 to 100 in steps of 10.

Track					Scan Speed	Laser Intensity		
No.	Mode	Start by	Condition	Interval		Ar	HeNe-G	HeNe-R
☑1	For	Normal	N = 10 To 100					
☑2	XY	Normal	1	0.4		N		
☑3	Next	Normal						

1. In Condition column to track No. 1, enter For statement “N = 10 To 100 Step 10”.
2. In track No. 2, specify the laser intensity value of the Ar laser as variable N.
3. In track No. 3, specify the turning point of repetition processing.
4. Click the <Start> button.

<Start> button

Acquire images by varying the laser intensity value of the Ar laser in 10% steps as shown below

	1st	2nd	3rd	4th	5th	…	10th time
Laser intensity %	10	20	30	40	50	…	100

1 Setting the Observation Condition

Before setting and starting the XYT observation for hours using the Protocol Processor, execute a repeated scan and set the condition for the observation.

Assuming FITC to Channel 2 and image size to 320X240 pixels, the following figure shows an example of image acquisition.

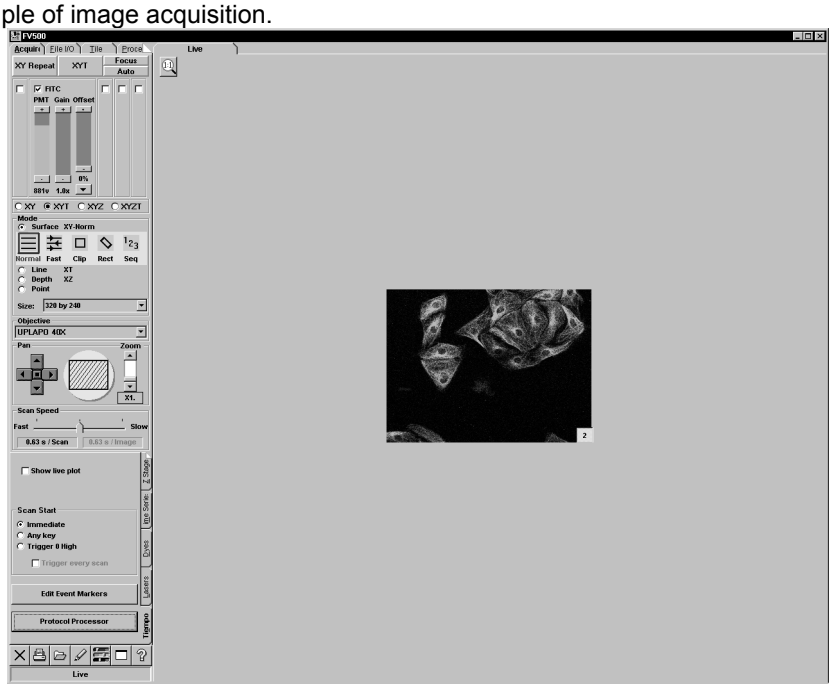


Fig. 2-59 Panel After a Repeated Scan

2 Editing the Protocol

Start and edit the protocol after setting of the observation condition has been completed.

For starting method, refer to section 2-4-1, “Starting the Protocol Processor”.

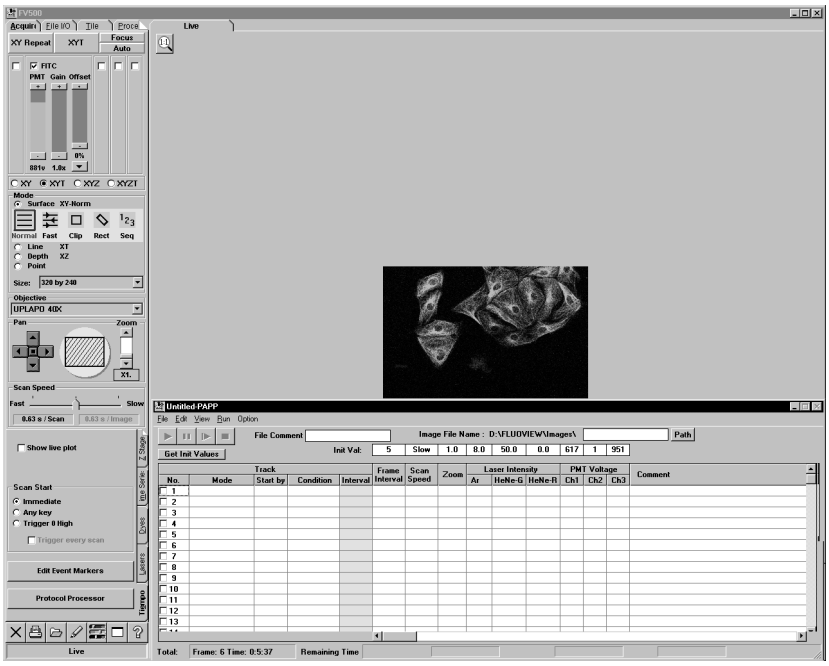


Fig. 2-60 Panel Displaying the [PAPP] window

Set the [PAPP] window as shown below;

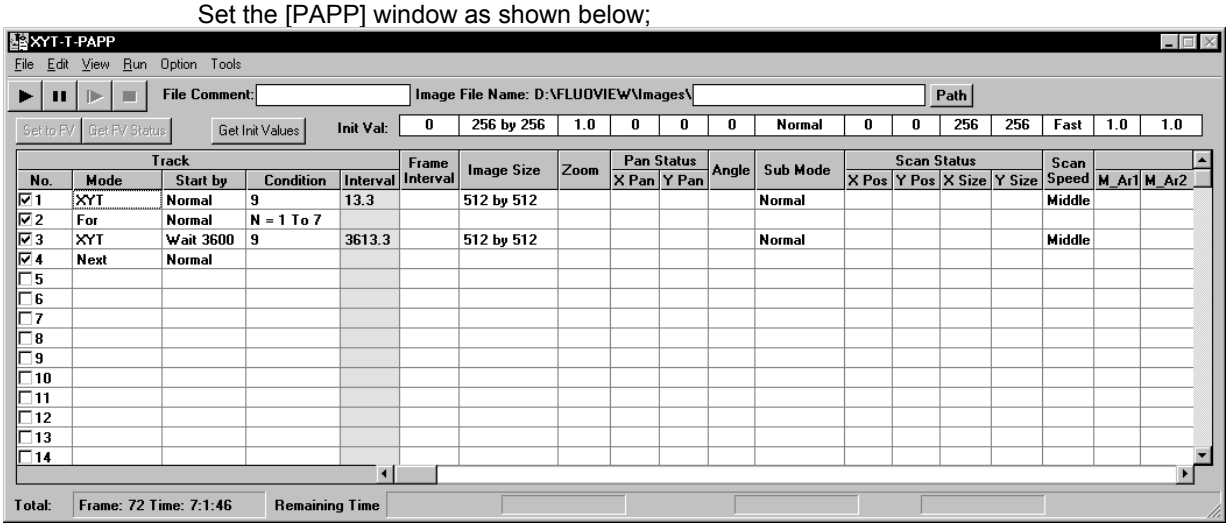


Fig. 2-61 [PAPP] window Setting an Example of Observation Condition

Set each Track as sown below;

- **Setting Track 1**

Acquire nine images in XYT observation.

1. Select “New mode”-“XYT” in [Mode] of [Track].
2. Select “Normal” in [Start by] of [Track].
3. Enter “9” to [Condition] of [Track].
4. Select “Middle” in [Scan Speed].
5. Enter the laser intensity value for image acquisition to [Laser Intensity].
6. Enter the PMT value of each channel to [PMT Voltage].
7. Enter image file name to be stored to [Save FileName.].
8. Enter a comment in [Comment] if necessary.

TIP

The actual image file name becomes using the name of [Image File Name] that followed by the acquisition order number.
Assign the image file name in the [Image File Name] text box area before protocol starts.

- **Setting Track 2**

Specifies N variable and designate range to be repeated. Protocol continues between the next Track of “For” statement and the Track just before “Loop” statement.

1. Select “For” in [Mode] of [Track].
2. Enter ”N = 1 To 7” to [Condition] of [Track].
3. It is not necessary to enter the values in other cells.

- **Setting Track 3**

  Acquire nine images at one-hour interval in each Track in XYT observation.

  1. Select “New mode”-“XYT” in [Mode] of [Track].
  2. Enter “Wait 3600” in [Stand by] of [Track]. (Enter the value by the second.)
  3. Enter “9” in [Condition] of [Track].
  4. Select “Middle” in [Scan Speed].
  5. Enter the laser intensity value for use in image acquisition in the cell for each laser in [Laser Intensity].

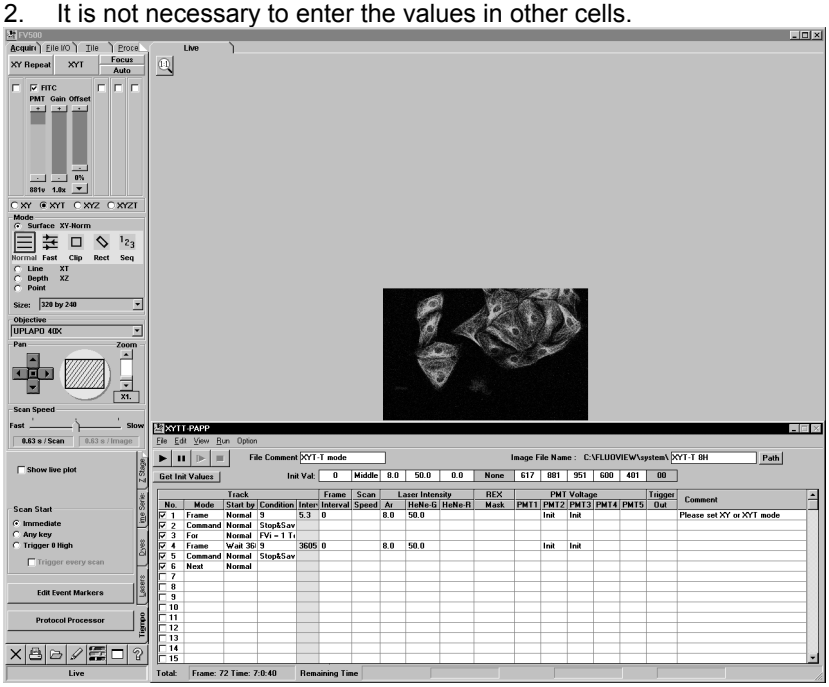
     Enter “8” in [Ar] and “50” in [HeNe-G].
  6. Enter the PMT value of each channel in the cell for each channel in [PMT Voltage].

     Enter “Init” in [PMT2] and [PMT3].
  7. Enter the image file name to be stored to [Save FileName.].

- **Setting Track 4**

  Specifies the repeated protocol by use N variable.

  1. Select “Next” in [Mode] of [Track].
  2. It is not necessary to enter the values in other cells.



One Point!

XYZT observation protocol becomes available when “New mode”-“XYZT” is selected instead of selecting “New mode” – XYT on [Mode] of [Track].

3 Saving the Protocol

Save the protocol edited.

The protocol can also be saved after being executed.

1. Select [Save] or [Save as] in the [File] menu in the [PAPP] window.

   Save using the dialog box displayed.

   For details, see section 2-4-3, “Saving the Protocol”.

4 Executing the Protocol

Execute the protocol assembled.

<Start> button

1. Click the <Start> button in the [PAPP] window.

   For details, see section 2-4-4, “Executing the Protocol”.

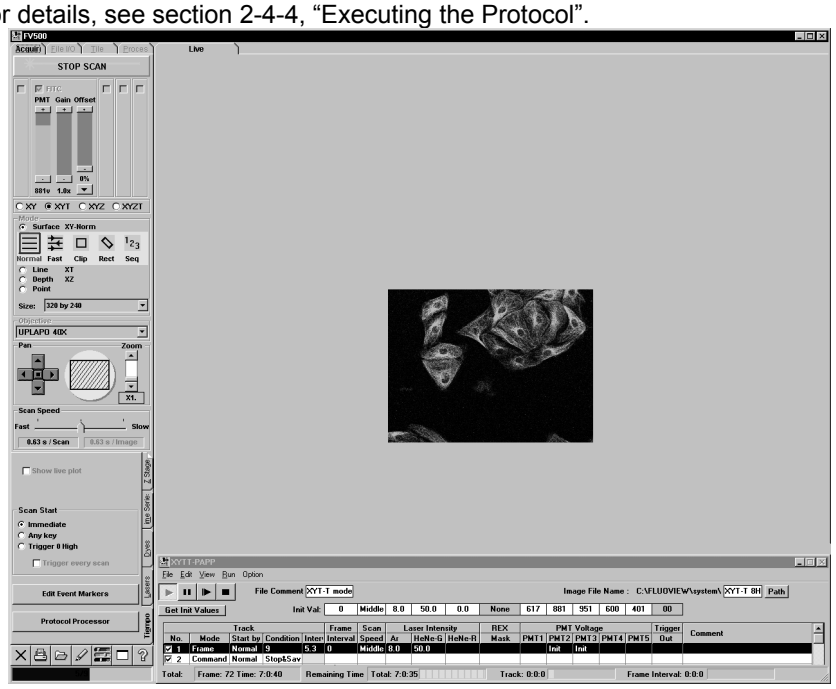


Fig. 2-62 Panel While Executing the Protocol

5 Exiting the Protocol

After the initial image has been acquired and saved, “saving the image acquired in Track 4 in Track 5” is repeated for 7 times, after which the protocol terminates.

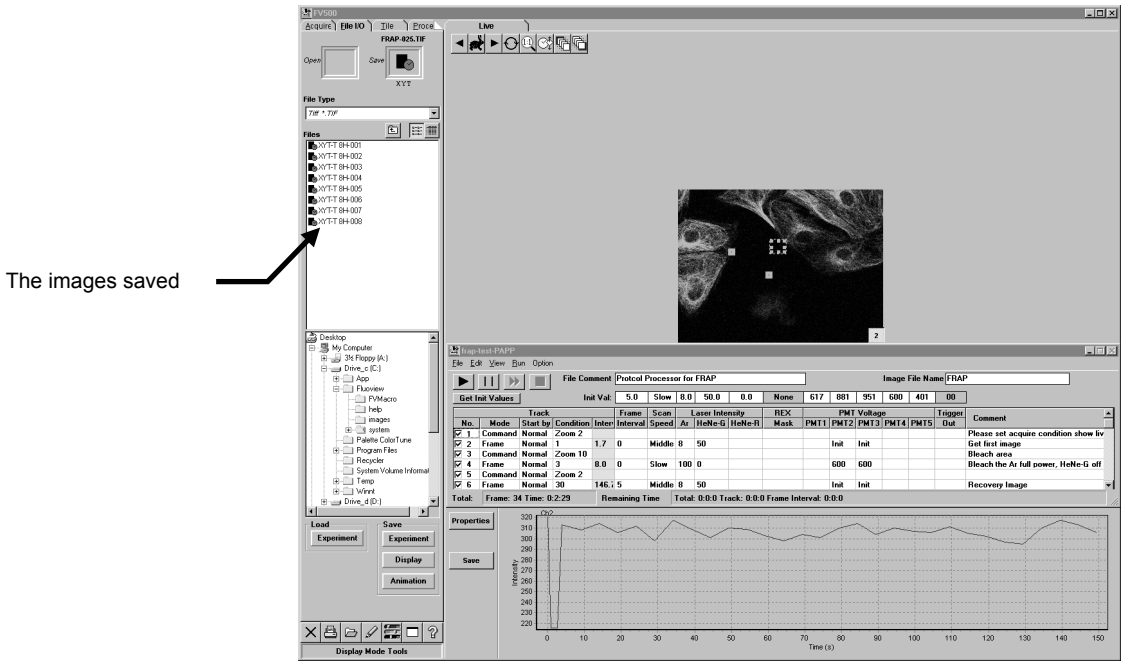
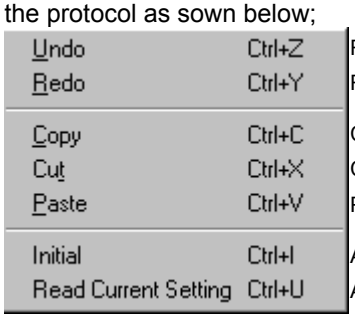


Fig. 2-63 Panel When Exiting the Protocol

2-4-9 Pop-up Menu

Right-clicking the mouse in the cells in the [PAPP] window displays the pop-up menu to edit the protocol as sown below;



Removes the effect of the previous operation.
Repeats the previous operation.
Copies the value in the selected cell.
Cuts the value in the selected cell.
Pastes the value copied or cut into another cell.
Applies the initial value into cell.
Applies the value in the [Acquire] panel into cell.

One Point!

In addition to the pop-up menu, the menu in the [PAPP] window is available to edit the protocol.

- **Remove the effect of the previous operation.**
  Select “Undo” in the [Edit] menu in the [PAPP] window.
- **Repeat the previous operation.**
  Select “Redo” in the [Edit] menu in the [PAPP] window.
- **Copy the value in cell.**
  Select “Copy” in the [Edit] menu in the [PAPP] window.
- **Cut the value in cell.**
  Select “Cut” in the [Edit] menu in the [PAPP] window.
- **Paste the value copied.**
  Select “Paste” in the [Edit] menu in the [PAPP] window.
- **Insert a cell.**
  Select “Insert” in the [Edit] menu in the [PAPP] window.
- **Insert the cell copied.**
  Select “Insert Copied Cell” in the [Edit] menu in the [PAPP] window.
- **Delete the value in cell.**
  Select “Delete” in the [Edit] menu in the [PAPP] window.

2-4-10 Restrictions of [PAPP] setting

2-4-10-1 Restrictions with [Mode] and [Sub Mode]

There are restrictions at Mode and its Sub Mode setting in [PAPP].
The following table shows the restriction.

Mode	Sub Mode	Scan Status Row			
		X Pos	Y Pos	X Size	Y Size
XY	Normal	X	X	X	X
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	Fast	X	X	X	X
	FastClip	O	O	O	O
	Seq_Normal	X	X	X	X
	Seq_Clip	O	O	O	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	X	X	X	X
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XYT	Normal	X	X	X	X
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	Fast	X	X	X	X
	FastClip	O	O	O	O
	Seq_Normal	X	X	X	X
	Seq_Clip	O	O	O	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	X	X	X	X
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XYZ	Normal	X	X	X	X
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	Seq_Normal	X	X	X	X
	Seq_Clip	O	O	O	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	X	X	X	X
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*

Mode	Sub Mode	Scan Status Row			
		X Pos	Y Pos	X Size	Y Size
XYZT	Normal	X	X	X	X
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	Seq_Normal	X	X	X	X
	Seq_Clip	O	O	O	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	X	X	X	X
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XT	Normal	X	O	X	O
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	FreeLine	X	X	X	X
	Fast	X	O	X	O
	FastClip	O	O	O	O
	LineSeq_Normal	X	O*	X	O*
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XZ	Normal	X	O	X	O
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	FreeLine	X	X	X	X
	LineSeq_Normal	X	O*	X	O*
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XZT	Normal	X	O	X	O
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	FreeLine	X	X	X	X
	LineSeq_Normal	X	O*	X	O*
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
Pt*T	Normal	O	O	O	O

O means can use the combination.
X means can not set
O* Input is possible when combiner is FV5-COMBA only.

2-5 Changing the Image Display Method

The method of displaying an image acquired by observation or opened from a file can be changed as described below.

2-5-1 Displaying an Image in Simulated Colors

<LUT> button

1. Display the image window of the image to be colored at the front.
2. Click the <LUT> button in the toolbar. The [Color Tool] dialog box appears.

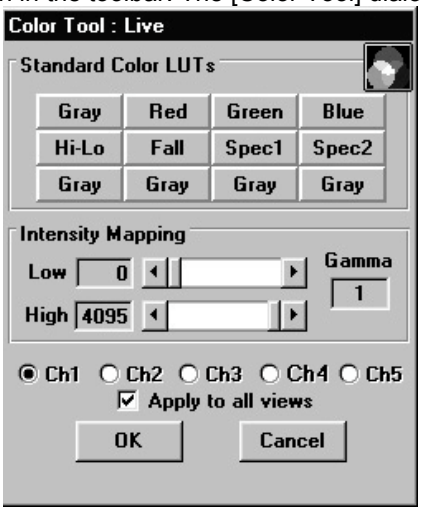


Fig. 2-64 [Color Tool] Dialog Box

3. When the image was acquired from more than one channel, select the channels to be colored using the option buttons among [Ch1] to [Ch6]. (The option buttons among [Ch1] to [Ch6] are displayed only when an image acquired from more than one channel mode is displayed (selected).
4. From the [Standard Color LUTs] group box, select the desired color button. The selected LUT will be applied immediately to the image in the image window.
5. Click the <OK> button.

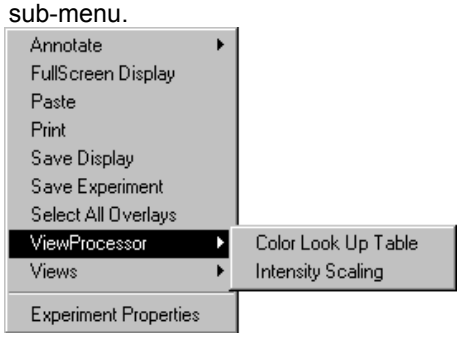
TIP

The [Apply to all view] check box can be selected while the image window showing multiple images created in the [Title] panel is displayed.
When this check box is checked, all changes are applied to all of the images shown in the image window.

One Point!

The [Color Tool] dialog box can also be displayed by a mouse operation.

1. Display the image to be colored at the front of the image window, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [View Processor] from the menu, then select [Intensity Scaling] from the displayed sub-menu.



2-5-2 Editing the LUT (Look Up Table)

An original LUT can be created by editing an existing LUT.

2-5-2-1 LUT Graph Editing According to Colors

<LUT> button

<Graph display> button

1. Display the image window of the image that you want to edit the LUT.
2. Click the <LUT> button in the toolbar. The [Color Tool] dialog box appears as shown in Fig. 2-64.
3. Click the <Graph display> button on the top right of the [Color Tool] dialog box. The dialog box shows the LUT intensity graph.
4. Select the LUT color to be edited by checking the check boxes below the graph in the [Color LUT Tool] group box.
5. Set the range of intensity graph application using the [Low] and [High] scales in the [Intensity Mapping] group box.

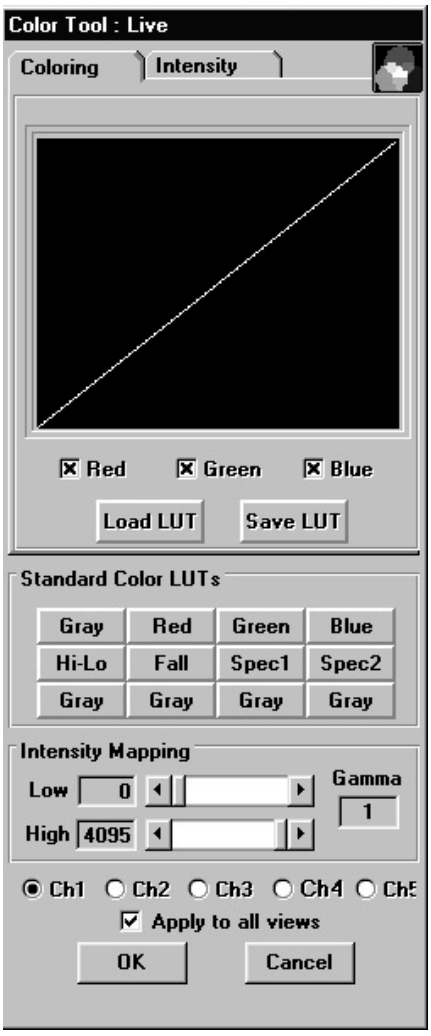


Fig. 2-65 LUT Intensity Graph Display

TIP

Double-clicked the [Low] scale, [High] scale and [Gamma] text box, they are reset to the default values (“0” with the [Low] scale, “4095” or “255” with the [High] scale and “1.0” with the [Gamma] text box).

6. The inclination of the graph in the [Color LUT Tool] group box can be changed by dragging an end of the graph line. The selected LUT will immediately be applied to the image in the image window.
7. If it is required to save the edited LUT in a file, click the <Save LUT> button in the [Color LUT Tool] group box.

TIP

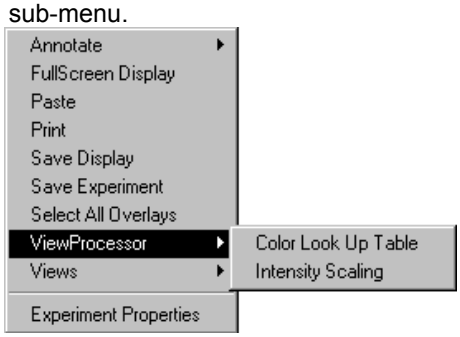
To load a previously saved LUT, click <Load LUT>.

8. Click the <Graph display> button at the top right of the [Color Tool] dialog box again.
9. Finally, click the <OK> button to exit from the LUT editing.

One Point!

The [Color Tool] dialog box can also be displayed by a mouse operation.

1. Display the image to be colored at the front of the image window, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [View Processor] from the menu, then select [Intensity Scaling] from the displayed sub-menu.



2-5-2-2 LUT Graph Editing by Gamma Correction

The intensity data of an image can be reallocated to make it easier to view.

<LUT> button

<Graph display> button

1. Display the image window of the image to be subjected to LUT change.
2. Click the <LUT> button in the toolbar. The [Color Tool] dialog box appears as shown in Fig. 2-64.
3. Click the <Graph display> button on the top right of the [Color Tool] dialog box. The intensity graph of the LUT appears in the [Intensity] dialog box.
4. Set the range of intensity graph application with the [Low] and [High] scales in the [Intensity Mapping] group box.

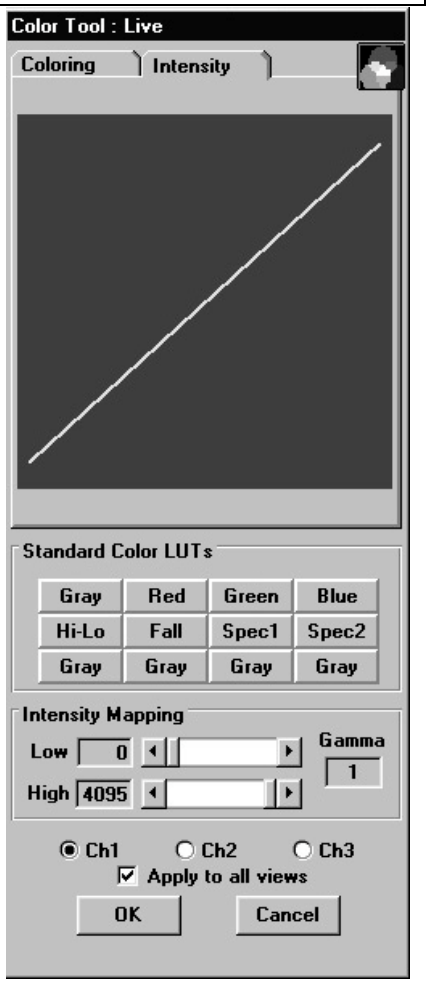


Fig 2-66 LUT Intensity Graph Display

TIP

Dragging one end of the graph makes it possible to change the inclination. The set intensity graph is immediately reflected in the image in the image window.

TIP

Double-clicked the [Low] scale, [High] scale and [Gamma] text box, they are reset to the default values (“0” with the [Low] scale, “4095” or “255” with the [High] scale and “1.0” with the [Gamma] text box).

5. The gamma value can be changed by dragging on the graph. The set intensity graph is immediately reflected in the image in the image window.

TIP

The gamma value can also be changed by entering a value in the [Gamma] text box in the [Intensity Mapping] group box.

6. If it is required to save the LUT in a file, click the <Save LUT> button in the [Color LUT Tool] group box.

TIP To load a previously saved LUT, click the <Load LUT> button.

2-5-3 Switching the Displayed Channels (Ch1 – Ch5)

The buttons on the bottom left of the screen can be used to select where the image of a single channel or images of multiple channels are to be displayed. For the simultaneous display of multi-channel images, see section 2-5-4, “Displaying Images of Multiple Channels Simultaneously”.

1. Display the image window of for the image obtained from multiple channels at the front.
2. Click the image to display the <Display channel switch> buttons on the bottom left of the image.
3. Select the channels which should be displayed by pushing <Display channel switch> buttons. The color of a selected channel's button becomes darker.
4. Press the previously pressed <Display channel switch> buttons to let them disappear.

<Display channel switch> buttons

<Channel 1> button

<Channel 2> button

<Channel 3> button

<Channel 4> button

<Channel 5> button

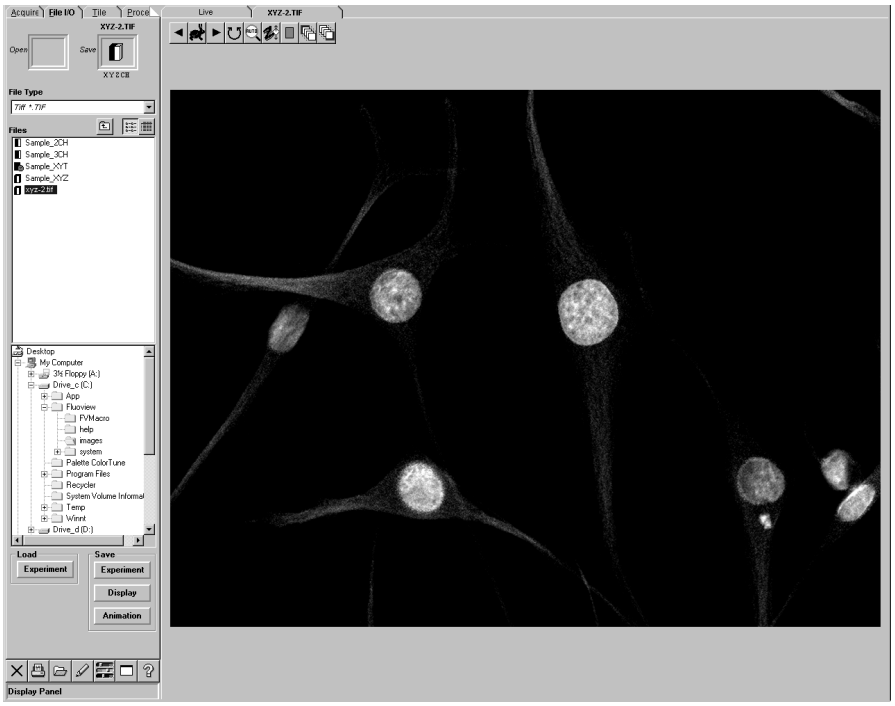


Fig. 2-67 Panel Displaying the Channel 2 Image

2-5-4 Displaying Images of Multiple Channels Simultaneously (Side By Side Views, Over And Under Views, Single View)

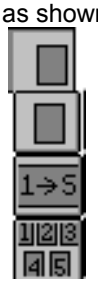
Images from multiple channels can be displayed either by merging them or placing them side by side. It is also possible to display the image of only one of these channels.

Use the buttons displayed at the top of the image window and those on the bottom right which are displayed when the corresponding image is clicked. For the display of the image of only one channel, see section 2-5-3, "Switching the Displayed Channels".

2-5-4-1 Displaying Images Separately Per Channel (Side By Side Views, Over And Under Views)

<Display switching> button

1. Display the image window of one of the images to be displayed side by side at the front.
2. Click the <Display switching> button at the top of the image window. The list of buttons as shown below appears.



<Side by side views> buttons

<Over and Under views> buttons

3. From the displayed list of buttons, click one of the <Side by side Views> button or <Over and under views> button. The icon shown in the <Display switching> button will change to the icons of the <Side by side views> button or <Over and under views> button.

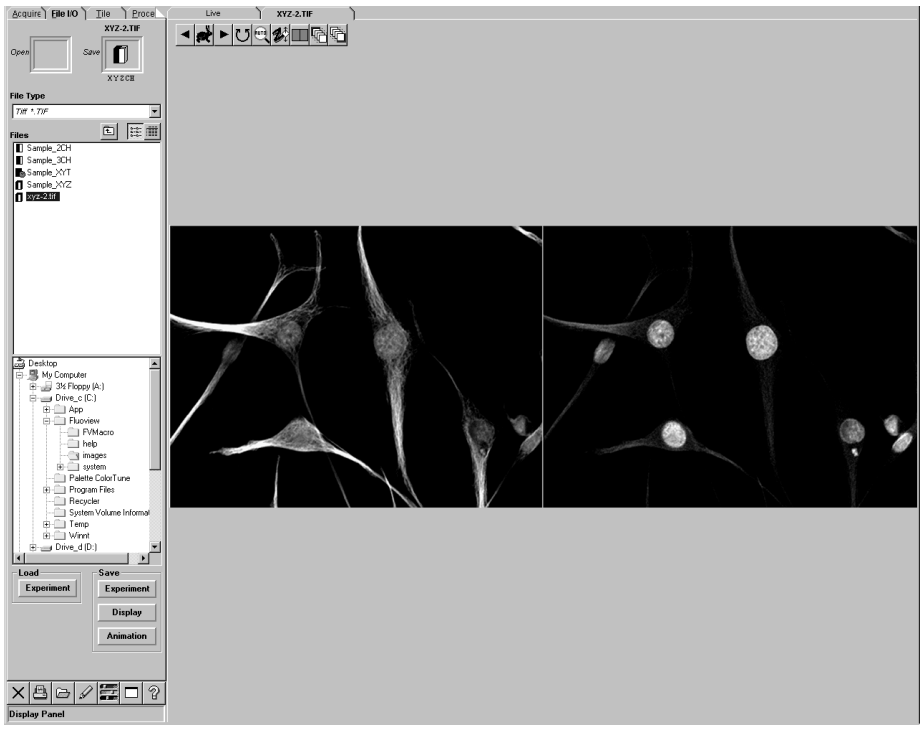
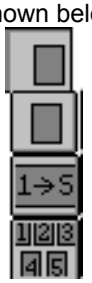


Fig. 2-68 Panel Displaying Images of Two Channels Side By Side

2-5-4-2 Displaying Merged Image of Multiple Channels (Single View)

<Display switching> button

1. Display the image window of the merged image of multiple channels at the front.
2. Click the <Display switching> button at the top of the image window. The list of buttons as shown below appears.



<Single View>button

3. From the displayed list of buttons, press the <Single View> button. The image window will show the images of multiple channels side by side. At the same time, the icon shown in the <Display switching> button will change to the icon of the <Single View> button.

<Display channel switch> buttons

4. Click the image to display the <Display channel switch> buttons at the bottom left of the image.

<Channel 1> button

<Channel 2> button

<Channel 3> button

<Channel 4> button

<Channel 5> button

5. Select the channels which should be displayed by pushing <Display channel switch> buttons. The color of a selected channel's button becomes darker.

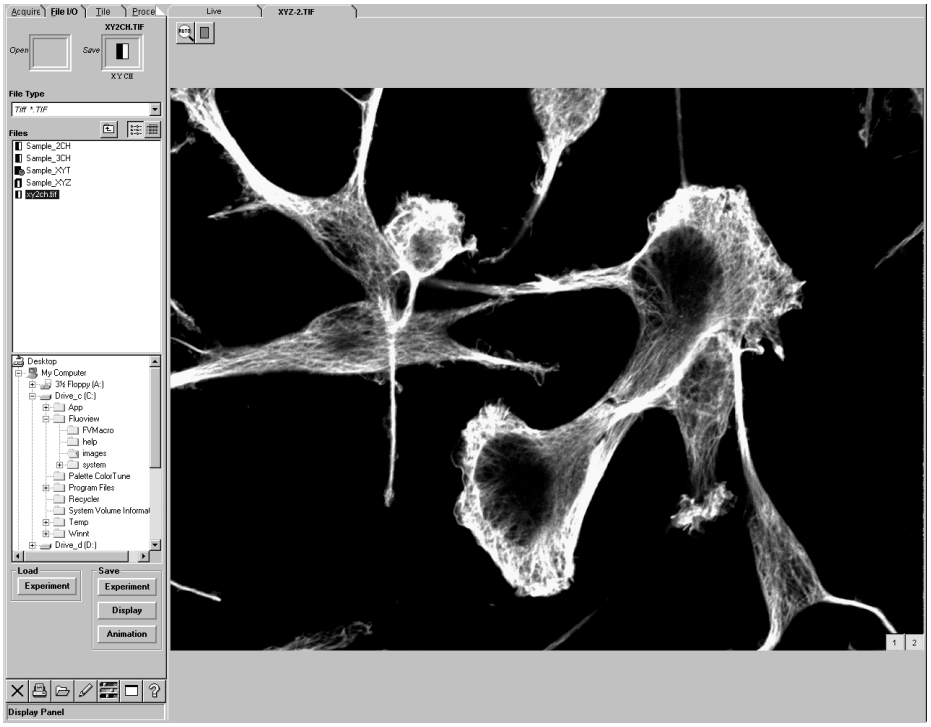


Fig. 2-69 Panel Displayed Merged Image of Two Channels

2-5-5 Changing the Number of Divided Images

The number of images viewed simultaneously can be changed.

TIP

Increased image is only to be displayed. The image increased in Add View is not subjected to these operations described below.

2-5-5-1 Increasing the Number of Divided Images

1. Display the image window of the image to be changed at the front.

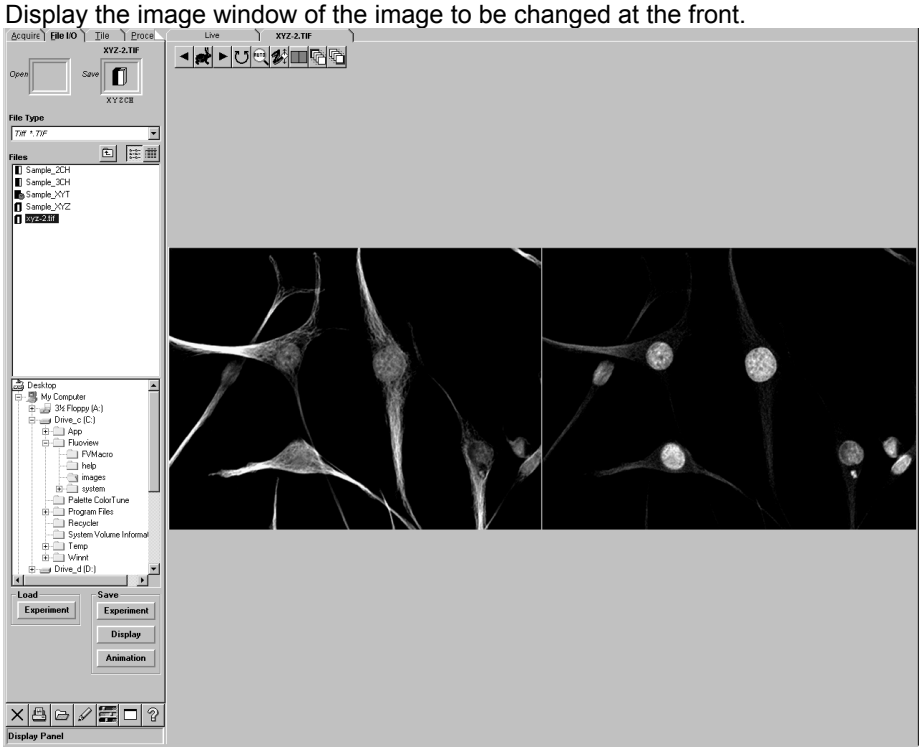
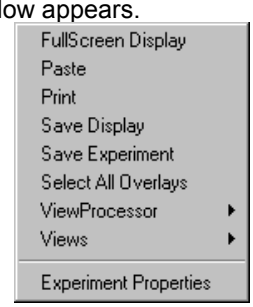


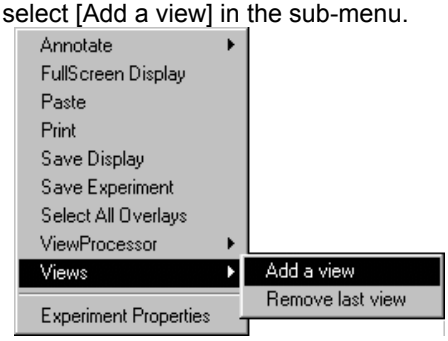
Fig. 2-70 Image window

2. Right-click the image.

   A pop-up menu as shown below appears.



3. Select [Views], then select [Add a view] in the sub-menu.



4. A view is added to the rightmost position on the image window.

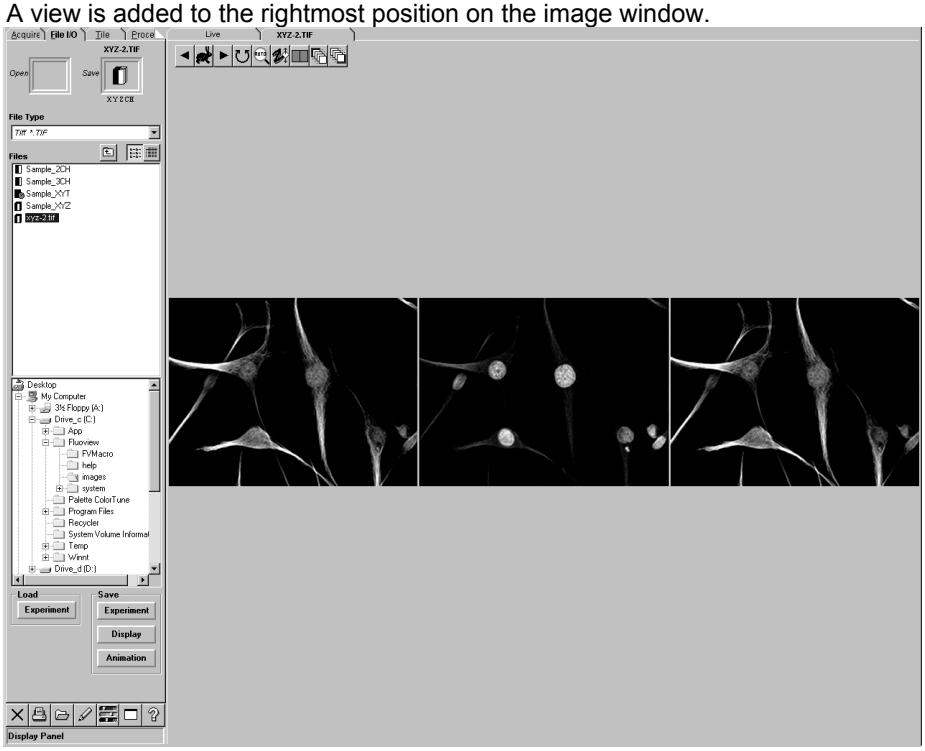


Fig. 2-71 Image window After View Addition

TIP Up to 6 views can be displayed at once.

2-5-5-2 Decreasing the Number of Divided Images

1. Display the image window of the image to be changed at the front.

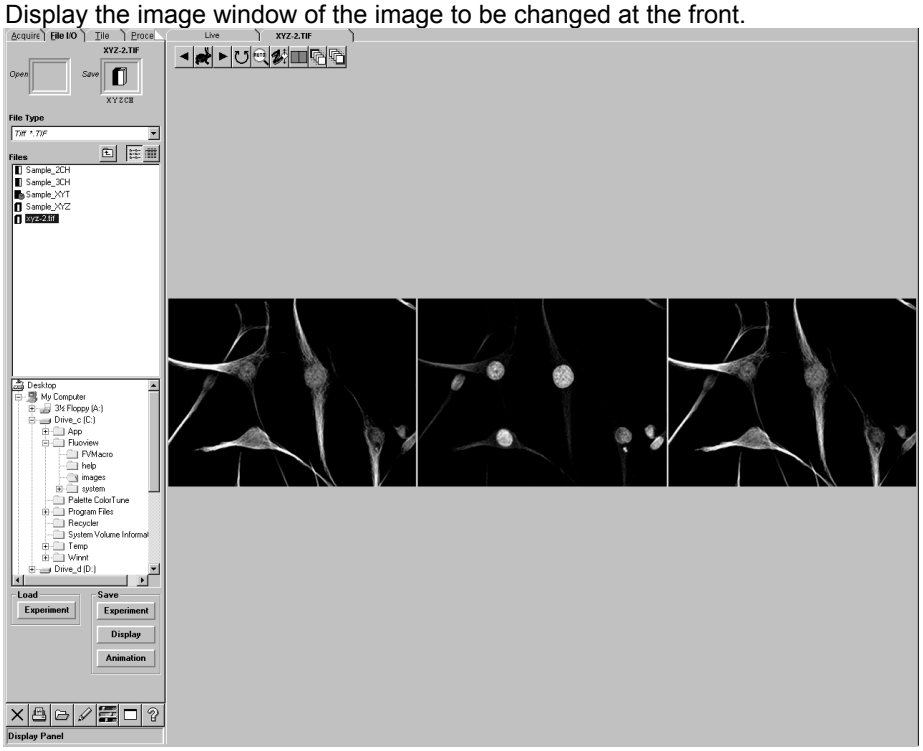
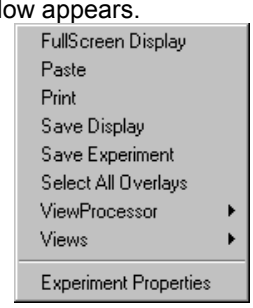


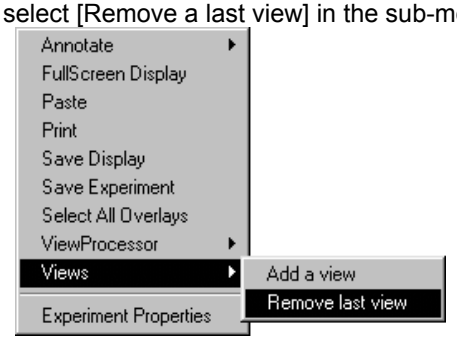
Fig. 2-72 Image window

2. Right-click the image.

   A pop-up menu as shown below appears.



3. Select [Views], then select [Remove a last view] in the sub-menu.



4. A view is removed from the image window.

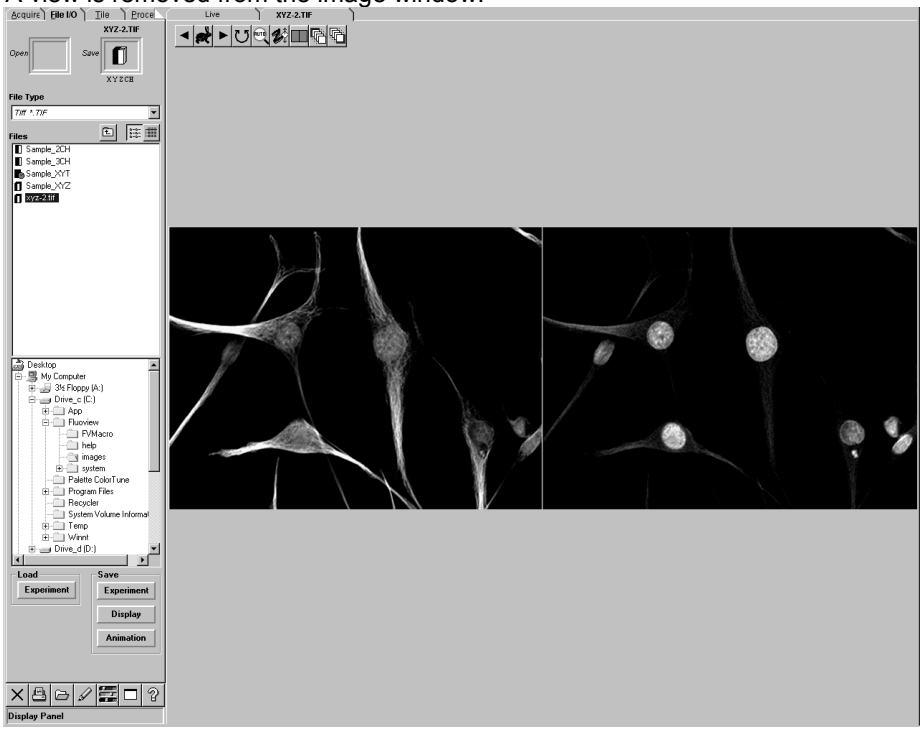


Fig 2-73 Image window after View Removal

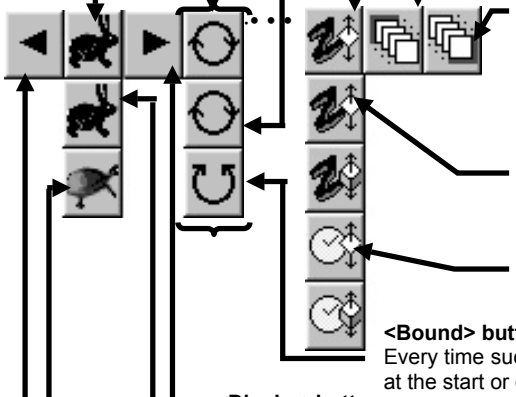
2-5-6 Switching the Display Method of Multiple Images

With images composed of multiple slices, such as time-lapse images or images acquired by changing the multiple sections, the image to be displayed at the front position can be switched or the images can be displayed successively.

1. Display the image window of the multiple images.
   The buttons as shown below are displayed at the top of the image window.

2. To switch the image to the image of another multiple sections, click the <Z/T series switch> button then, from the displayed list of buttons, click the <XYZ series> button.
   To switch the image to the image of another moment in the elapsed time, click the <Z/T series switch> button then, from the displayed list of buttons, click the <XYT series> button. (The icon in the <Z/T series switch> button will change to the icon of the selected button.)

3. Display the image to be displayed at the front by using the <Display> buttons at the top of the image window.



<Loop> button
Successive display or frame-by-frame display is repeated in the same direction.

<Loop/Bound switch>button

<Display speed switch>button
Set the speed of successive display.

<Z/T series switch> button

<Set start position> button
When successive display or frame-by-frame display is required, set the image with which the display should start. (“0” is set when this button is not used.)

<Set end position> button
When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, “n-1” is set when this button is not used.)

<XYZ series> button
Displays one of multiple images by selecting it according to the multiple sections (Z).

<XYT series> button
Displays one of multiple images by selecting it according to the time (T).

<Bound> button
Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.

<Display>button
Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame

<Rabbit>button
Press for successive display of image slices without pause.

<Turtle>button
Press for successive display of image slices at an interval of 0.8 sec

<Display>button
Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

TIP

Click and hold the <Display> button for successive display. To stop it, click the <Display> button again.

For frame-by-frame display, simply click the <Display> button.

2-5-7 Displaying Multiple Image Slices Together

With images composed of multiple slices, such as time-lapse images or images acquired by changing the multiple sections, the image slices can be displayed together for simultaneous viewing. However, note that the size per image reduces when the number of displayed image slices increases.

Use the [Tile] panel for displaying images together.

Display the [Tile] panel.

Icon of the displayed images (image slices shown together).

[Tiling] group box

Shows how the images are displayed in the image window.

[Columns] text box

Sets the number of columns, or the number of image slices displayed in a horizontal row.

[Experiment]

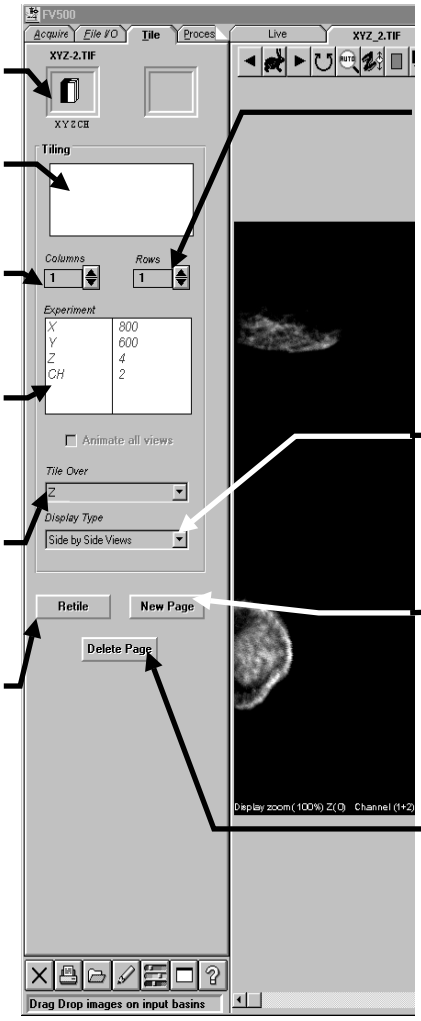
Shows the acquisition parameters used in acquisition of the displayed images.

[Tile Over] drop-down list

Select the acquisition parameter to be based on when arranging the images. [Self], [Z] or [T] can be selected.

<Retile> button

Displays the images by arranging them in the currently displayed image window.



[Rows] text box

Sets the number of rows, or the number of image slices displayed per vertical column.

[Display Type] drop-down list

Select the display method. [Single View], [Side by Side Views] or [Over and Under Views] can be selected.

[New Page] button

Displays images by arranging them in a new image window.

[Delete Page] button

Deletes the image window being displayed.

Fig. 2-74 [Tile] panel

<Change to tiled or multiplane view > button

One Point!

[Tile] panel moved to [Display Options] dialog box.

Select <Change to tiled or multiplane view> button on tool bar and bring [Display Options] dialog box to appear.

2-5-7-1 Displaying Multiple Images Per Channel

1. Display the image window of one the images which are to be displayed together. The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.

2. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.

3. When there are multiple images to be displayed, select the following items in the [Tile Over] drop-down list.
   - Self: The same images as the image being displayed will be displayed.
   - Z: Images are displayed according to change in multiple sections.
   - T: Images are displayed according to change in time.

4. Select the display method from the [Display Type] drop-down list.

5. Click the <New Page> button. A new image window appears showing the images displayed per channel.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed image window.**

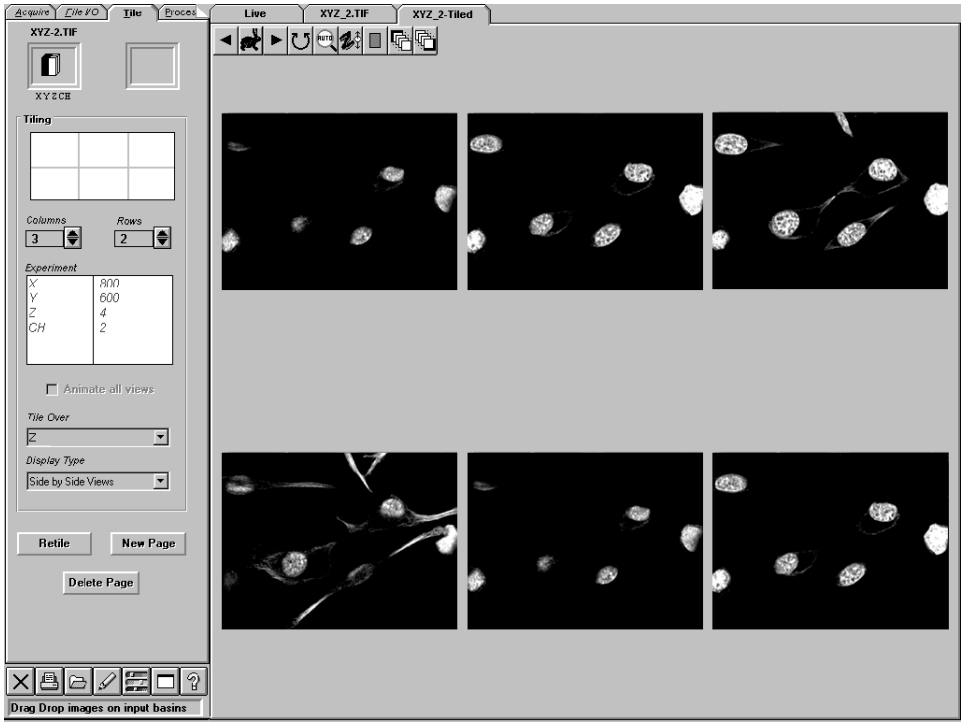


Fig. 2-75 Panel Displaying Images Per Channel

2-5-7-2 Displaying Images of Two Channels Together

Images acquired in a multi-channel mode can be displayed together for simultaneous view. The operation method is identical to the method for displaying images per channel except for the following point. See section 2-5-7-1, “Displaying Multiple Images Per Channel”.

- With images acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.

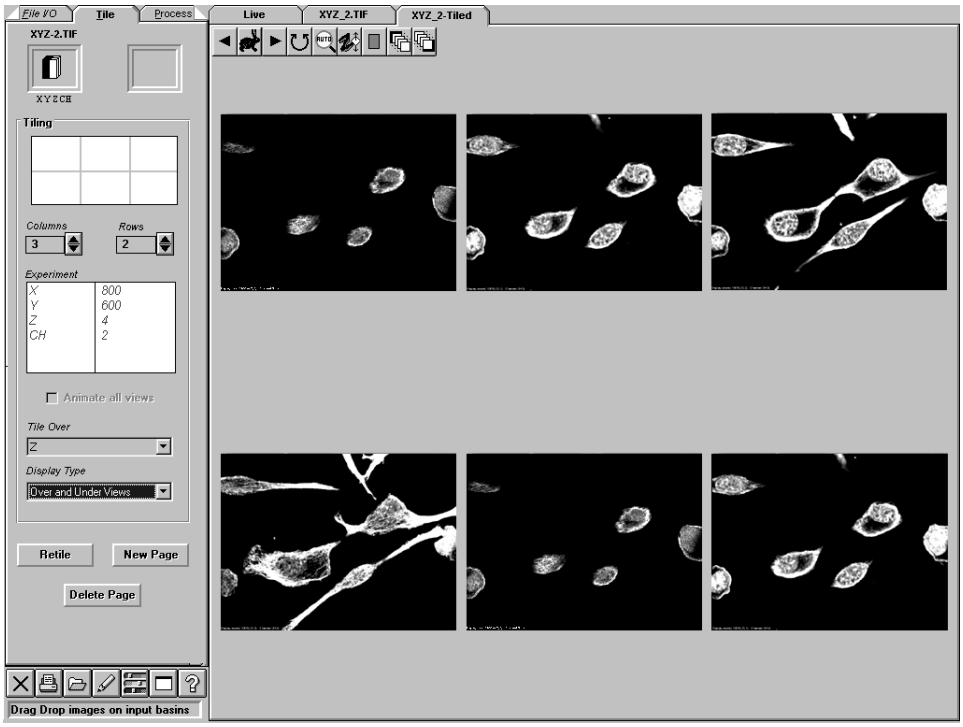


Fig. 2-76 Panel Displaying Images by Overlaying Different Channels

2-5-7-3 Displaying Time-Lapse Images

Multiple images acquired over time can be displayed side by side for simultaneous view.

1. Display the image window of one of the time-lapse images to be displayed together.
2. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. Select [T] from the [Tile Over] drop-down list.
4. When the time-lapse images were acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.
5. Click the <New Page> button. A new image window appears showing the images displayed per channel.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed image window.**

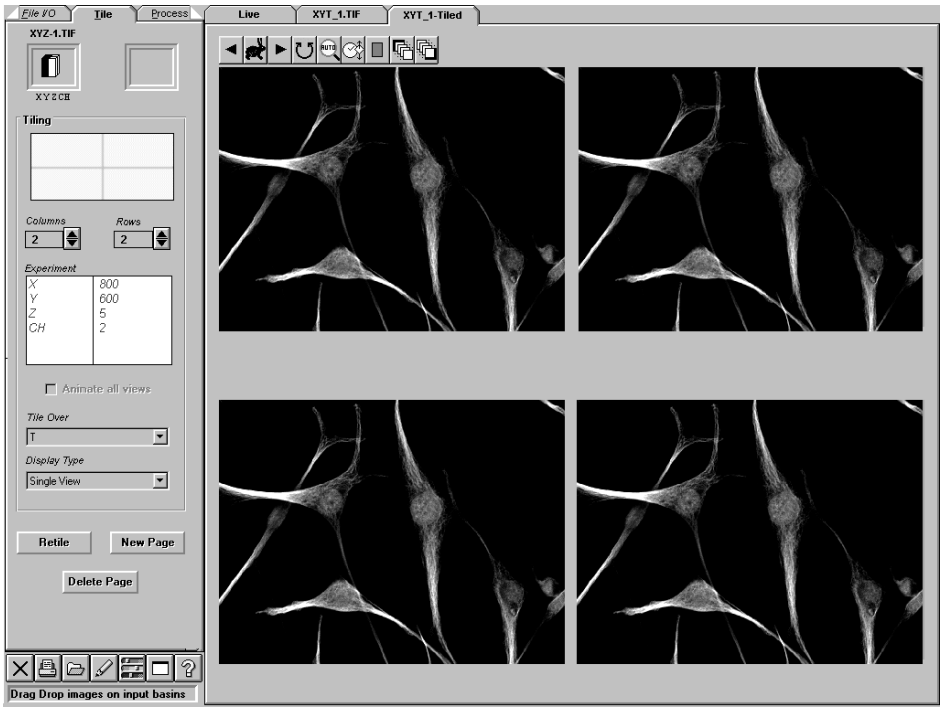


Fig. 2-77 Panel Showing Time-Lapse Images Together

2-5-7-4 Displaying Multiple Multiple sections Images

Images acquired from different multiple sections can be displayed together for simultaneous view.

The operation method is identical to the method for displaying time-lapse images except for the following point. See section 2-5-7-3, “Displaying Time-Lapse Images”.

- Select [Z] from the [Tile Over] drop-down list.

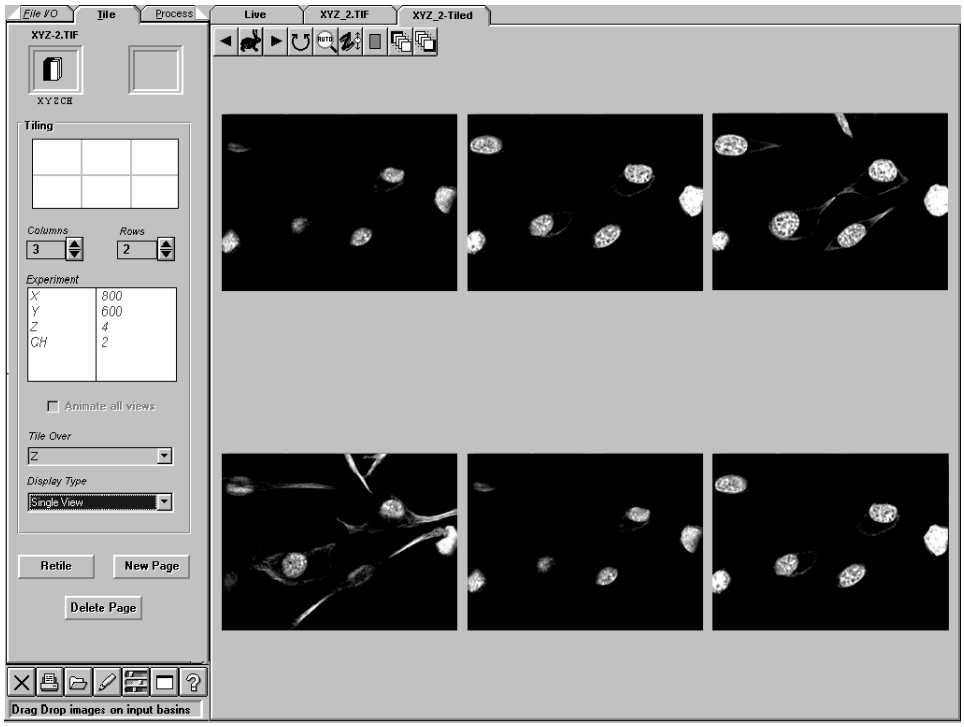


Fig. 2-78 Panel Displaying Different Multiple sections Images Together

2-5-7-5 Displaying Same Images in Different Display Methods

Images composed of multiple image slices can be displayed in more than one display methods together.

1. Display the image window of one of the images (image composed of multiple slices) to be displayed together.

   The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.

2. Set the number of images to be displayed together by using the <▲> or <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.

3. Select [Z] or [T] from the [Tile Over] drop-down list.

4. Click the <New Page> button. A new image window appears showing the same images as the displayed image. The number of the displayed images is as set in step 2 above.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed image window.**

5. Click the top left image of the displayed images.
   Buttons appear above the clicked image.

6. Switch the image display using the displayed buttons.

TIP For details on the display switching method, see sections 2-5-3, “Switching the Displayed Channels”, 2-5-4, “Displaying Images of Multiple Channels Simultaneously” and 2-5-6, “Switching the Display Method of Multiple Images”.

7. Perform steps 5 and 6 above for each of the displayed images.

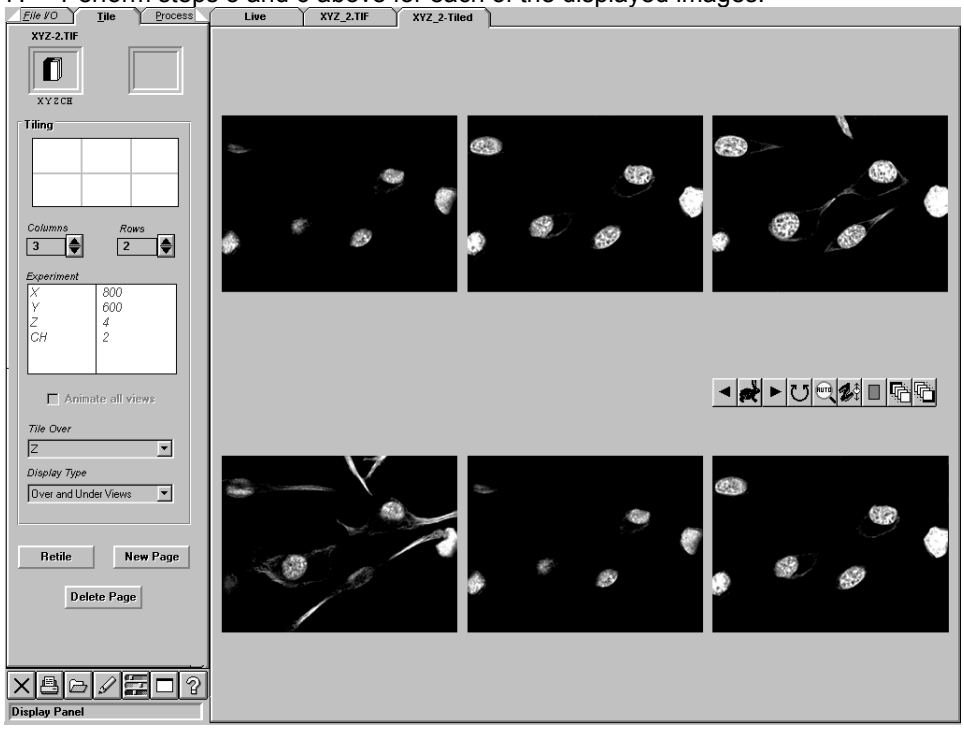
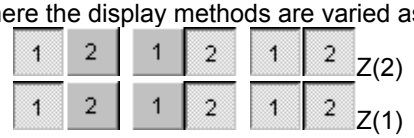


Fig. 2-79 Panel Showing 2-Channel Mode Images in Different Display Methods

Fig. 2-79 shows a panel where the display methods are varied as shown below.



2-5-7-6 Re-arranging Images Using the Same Display Method

All of the images displayed together in a image window can be rearranged simultaneously based on the same display method (channels, magnification, scroller position).

1. Click one of the images displayed together.
   Two sets of buttons appear above and below the clicked image.
2. Change the image display method using the displayed buttons.
3. Click the <Retile> or <New Page> button. All of the images in the panel are re-displayed using the same display method as that set in step 2 above.

2-5-7-7 Displaying Different Images Together

Two completely different images can be displayed together.
The two images to be displayed together should be acquired by observation or loaded by opening a file. If two images are not available, prepare them by image acquisition or file opening.

1. Display the image window of either image to be displayed with another image.
   The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.

<Experiment List> button

2. Click the <Experiment List> button in the toolbar. The [Experiments in Memory] dialog box appears as shown below.

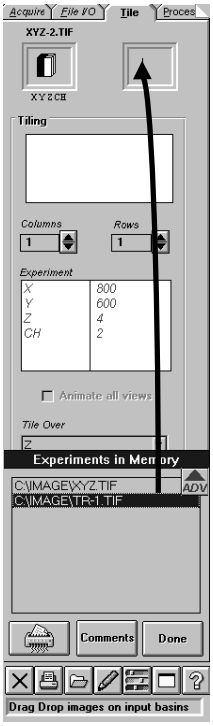


Fig. 2-80 [Experiments in Memory] Dialog Box

3. From the [Experiments in Memory] dialog box, select the file name of the second image to be displayed and drag it into the frame at the top right of the [Tile] panel. The icon of the second image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in the image acquisition are displayed in the [Experiment] panel.

TIP The mouse pointer turns into the image icon during dragging.

4. Click the <Done> button in the [Experiments in Memory] dialog box to close it.
5. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
6. When there are multiple images to be displayed, select the following items in the [Tile Over] drop-down list.
   - Self: The same images as the image being displayed will be displayed.
   - Z: Images are displayed according to change in multiple sections.
   - T: Images are displayed according to change in time.

7. When displaying images acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.
8. Click the <New Page> button. A new image window appears showing the two images one above the other.

   The image of the file displayed in the frame at the top left of the [Tile] panel is displayed on the upper part of the image window, and that of the file displayed in the frame at the top right is displayed on the lower part.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed image window.**

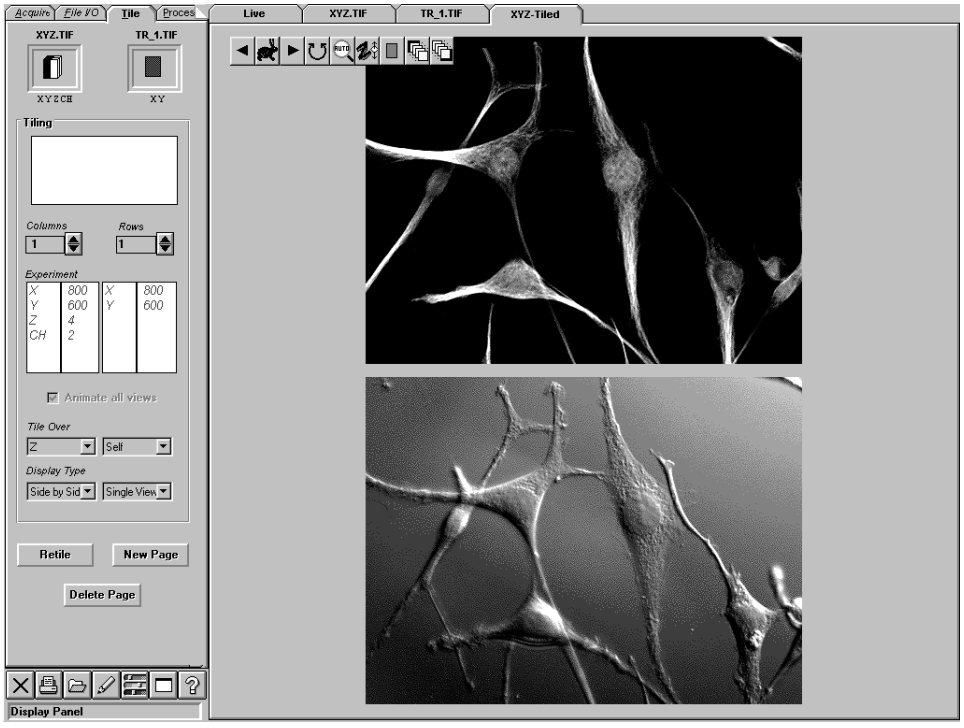
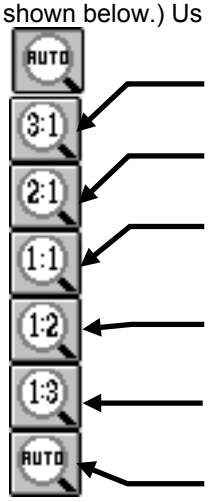


Fig. 2-81 Panel Displaying Two Different Images Together

2-5-8 Magnifying/Reducing an Image

The image can be magnified or reduced using the buttons displayed at the top of the image window. Magnification or reduction up to 3:1 or 1:3 the original image is possible.

1. Display the image window of the image to be magnified or reduced.
2. The buttons as shown below are displayed on the top of the image window. (Usually, the <Auto> button is displayed, and clicking it displays the list of buttons shown below.) Us these buttons to magnify or reduce the image.



<3:1> button
Magnifies the original image by 3 times.

<2:1> button
Magnifies the original image by 2 times.

<1:1> button
Displays the image in the original size.

<1:2> button
Reduces the original image by 1/2.

<1:3> button
Reduces the original image by 1/3.

<AUTO> button
Magnifies or reduces the image according to the current image window size.

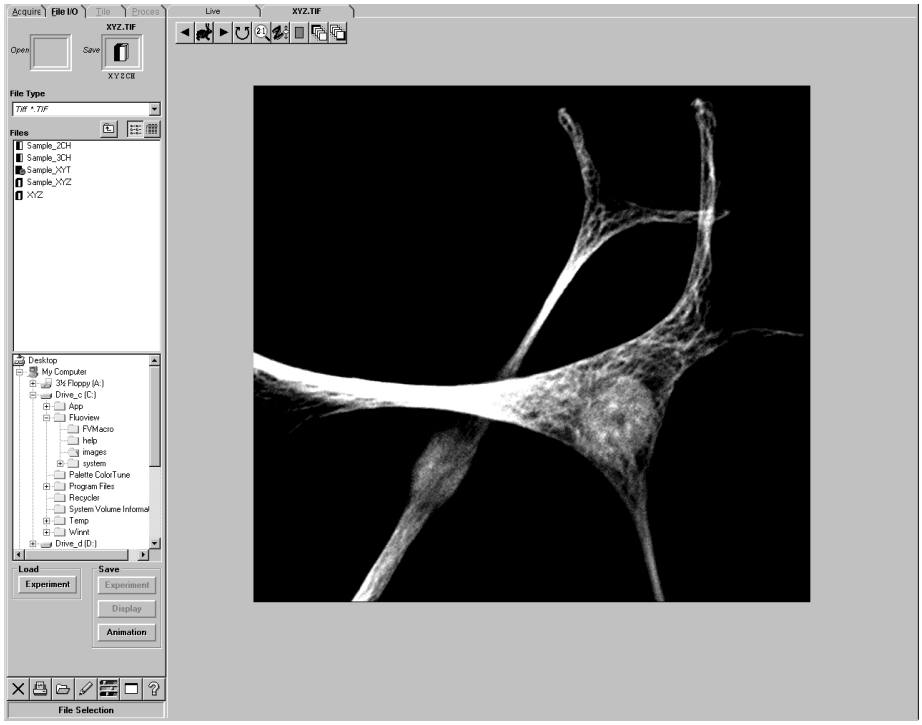


Fig. 2-82 Panel Showing a 2:1 Magnified Image

2-6 Image Processing

Images can be processed using the [Process] panel. Display the [Process] panel at the front.

2-6-1 Filtering

Use the [Filters] sub-panel in the [Process] panel to apply filtering to images.

1. Display the [Filters] sub-panel in the [Process] panel at the front.

Icon of the image being displayed (Image to be processed by filtering).

[Smoothing Filters] group box
Provides a command button for each noise filter.

[Sharpening Filters] group box
Provides a command button for each of the contour enhancement and image sharpening filters.

[DIC Image Filter] group box
Used for correction of DIC level irregularities.

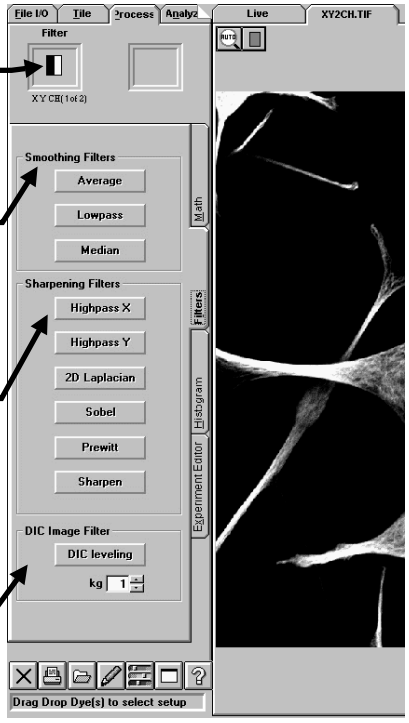


Fig. 2-83 [Filters] Sub-panel

2-6-1-1 Contour Enhancement

When an image is blurred by the boundaries between image grains becoming unclear, it can be sharpened by applying contour enhancement. Five types of filters are provided for use in the contour enhancement.

1 Laplacian filter

This filter enhances the contours of the image grains. If the image contains noise, the noise is also enhanced. By adding the original image to the image processed with Laplacian filtering, it is possible to obtain an image with stronger contour enhancement. The filter format is as shown below.

$$\begin{pmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{pmatrix}$$

<Set start position> button

<Set end position> button

<Display>button

<Display channel switch> buttons

<2D Laplacian> button

1. Display the image window of the image to be processed with Laplacian filtering.
2. When the image is composed of multiple image slices, the range of image slices to be filtered can be specified using the <Set start position> and <Set end position> buttons above the image. First display the image slice to start filtering using the <Display> button and click the <Set start position> button. Then, set the image slice to end filtering in the same way as above.
3. When the image to be filtered was acquired in the multi-channel mode, filtering is applied only to the channels being displayed.
   Example) When only the Ch1 image is displayed, filtering is applied to the Ch1 image only.

For the switching of channels, see section 2-4-3, “Switching the Display Channels”.

4. Click the <2D Laplacian> button. A new image window having the page tab named [Filter] appears, showing the filtered image.

During filtering, the status bar shows the progress of processing.

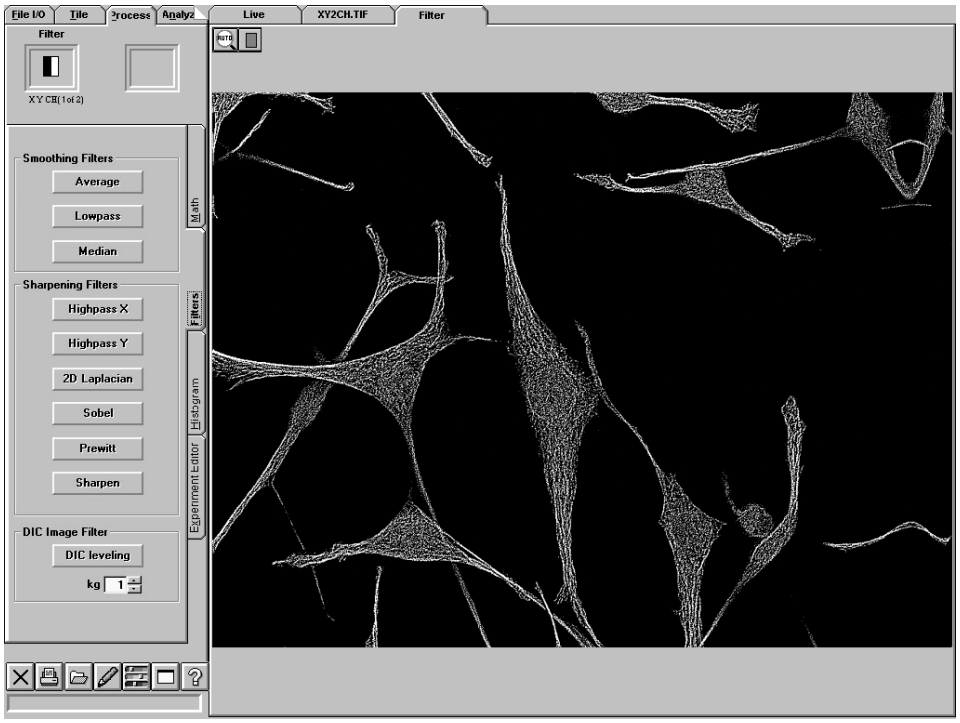


Fig. 2-84 Panel Displaying the Laplacian Filtered Image

2 Sobel filter

This filter enhances the contours of the image grains. If the image contains noise, the noise is also enhanced. It has two filter formats, X and Y, as shown below. The format providing the larger value after filtering is used.

$$\begin{pmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{pmatrix} \qquad \begin{pmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Sobel> button

- Click the <Sobel> button.

3 High-pass X filter

The HIGH-PASS X filter passes the high-frequency structures in the X-direction of image. In this way, it can extract details by detecting positions with large variation. This processing is useful for making structures clear or extract the edges. The filter format is as shown below.

$$\begin{bmatrix} -1 & -1 & -1 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \end{bmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Highpass X> button

- Click the <Highpass X> button.

4 Highpass Y filter

The HIGH-PASS Y filter passes the high-frequency structures in the Y-direction of image. In this way, it can extract details by detecting positions with large variation. This processing is useful for making structures clear or extract the edges. The filter format is as shown below.

$$\begin{bmatrix} 1 & 0 & -1 \\ 1 & 0 & -1 \\ 1 & 0 & -1 \end{bmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Highpass Y> button

- Click the <Highpass Y> button.

5 Prewitt filter

This filter enhances the contours of image grains in a similar way to the Sobel filter, but more strongly than it. It has two filter formats, X and Y, as shown below. The format providing the larger value after filtering is used.

X

-2	-2	-2
0	0	0
2	2	2

Y

-2	0	2
-2	0	2
-2	0	2

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Prewitt> button

- Click the <Prewitt> button.

2-6-1-2 Noise Reduction

When random noise interferes with an image, its irregularity increases and it become harder to see. Such noise can be reduced by means of filtering. Three kinds of filters are available for noise reduction.

1 Averaging filter

The averaging filter is used to eliminate details in image or reduce noise. However, as it makes everything in an image smooth, it also makes the edge sections dull, which sometimes result in the image resolution deterioration. The filter format is as shown below.

1	1	1
1	1	1
1	1	1

X 1/9

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Average> button

- Click the <Average> button.

2 Median filter

The Median filter reduces noise in image while leaving the edges intact. However, it may be ineffective in case noise is concentrated in some positions or the image is very noisy.

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Median> button

- Click the <Median> button.

3 Low-pass filter

The low-pass filter passes the low-frequency structures. In this way, it can eliminate small grains and provide smooth, noise-reduced image. filter format is as shown below.

$$\begin{pmatrix} 1 & 2 & 1 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{pmatrix} \text{ X } 1/16$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Lowpass> button

- Click the <Lowpass> button.

2-6-1-3 Image Sharpening

1 Sharpen filter

The sharpen filter turns blurred image into a clear image. The filter format is as shown below.

$$\begin{pmatrix} 0 & -1 & 0 \\ -1 & 5 & -1 \\ 0 & -1 & 0 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, “Laplacian filter”.

<Sharpen> button

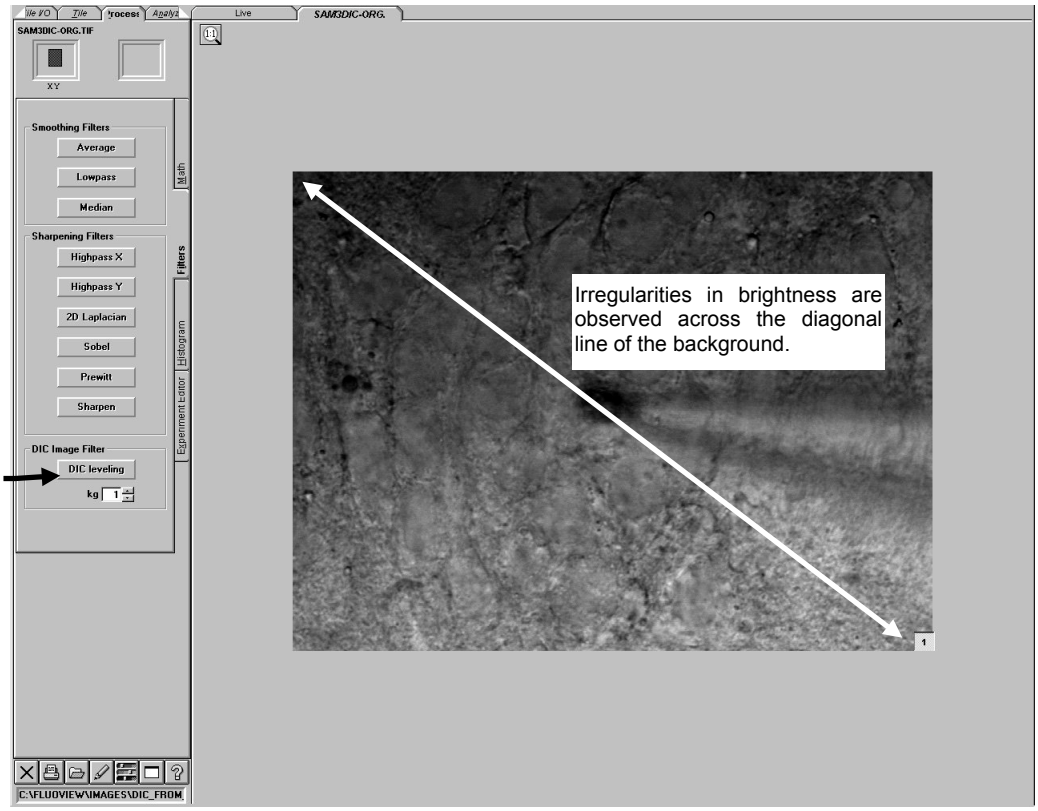
- Click the <Sharpen> button.

2-6-1-4 DIC Correcting DIC Level Irregularities

DIC level irregularities refers to uneven brightness of the image background which may be observed when a transmitted image is acquired in IDC observation.

The DIC level irregularities can be corrected to make the image easier to view.

1. Display the [Filters] sub-panel in the [Process] panel.



<DIC leveling>button
Automatic correction of irregularities of the image background

Fig 2-85 [Filters] Sub-panel

2. Enter the appropriate correction factor for the image background in the [kg] text box.

TIP

The correction factor can be set between 0.1 and 1 in steps of 0.1.

The standard factor is 1. If the effect is extreme, try using 0.5.

3. Click the <DIC leveling> button.

   The [Level] panel is newly created in the image window and shows an image after the correction of the DIC level irregularities of the background.

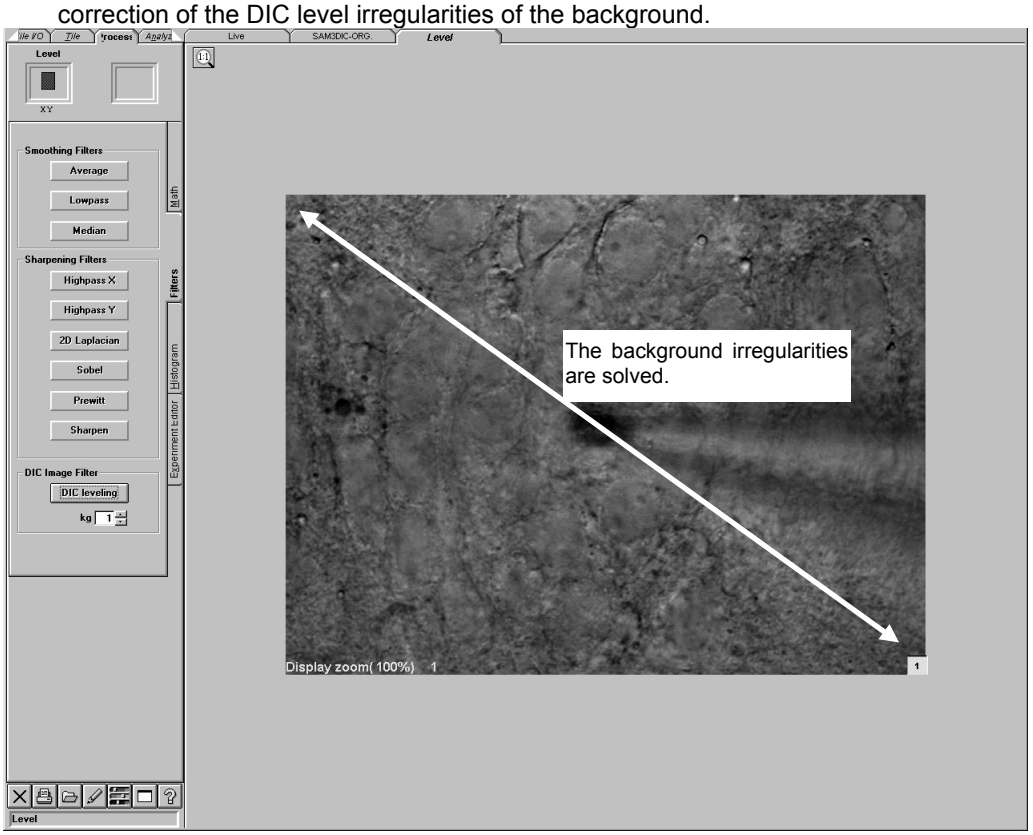


Fig. 2-86 [Level] Panel After DIC Level Correction

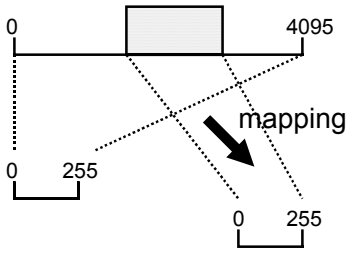
4. If the DIC level irregularities cannot be reduced, repeat steps 1 to 3 above by varying the correction factor.

2-6-2 Contrast Conversion

The LUT intensity can be mapped (re-assigned) while observing a histogram.

Mapping (re-assignment) results in changing the image contrast.

An image acquired by observation contains intensity information in values from 0 to 4095, but the intensity information used in actual display takes values from 0 to 255 by assigning the original values from 0 to 4095 to values from 0 to 255 usually. This facility changes the contrast by noticing a certain section between 0 and 4095 and mapping this section to values between 0 and 255.



1. Display the [Process] panel at the front.

2. Display the [Histogram] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to contrast change. The icon of the image is displayed here.

[Channel] option button
Select the channel to be subjected to LUT intensity mapping (re-assignment).

<Compute Histogram> button
Displays the histogram.

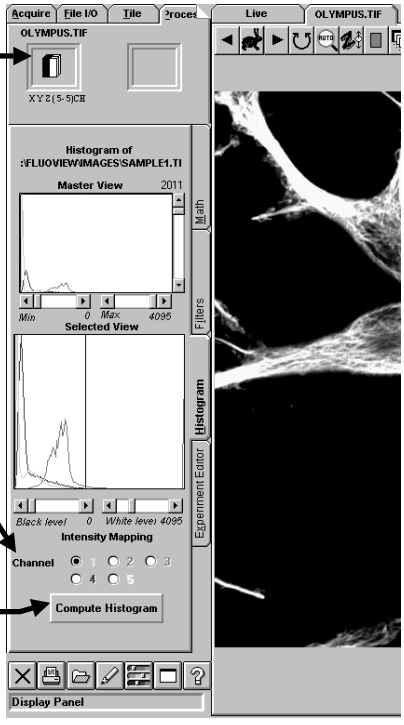


Fig. 2-87 [Histogram] Sub-panel

3. Display the image window of the image you want to change the contrast.
   The icon of the image is displayed in the frame at the top left of the [Process] panel

<Display channel switch> buttons

4. When the image was acquired in the multi-channel mode, select whether the LUT intensity mapping (re-assignment) is applied to multiple channels simultaneously or to a single channel.
   To select the target channel(s), use the <Display channel switch> buttons. The histogram of the selected channel(s) is displayed.
   Example) When only the Ch1 image is displayed, the histogram of Ch1 is displayed and mapping of only the Ch1 image is possible.

TIP For the switching of channels, see section 2-4-3, “Switching the Display Channels”.

5. Click the <Compute Histogram> button. A histogram appears as shown below.

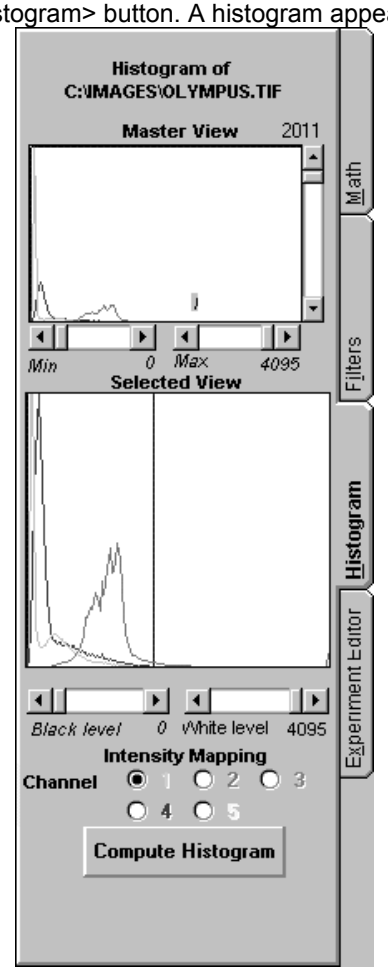


Fig. 2-88 Histogram

6. Enclose the histogram section of interest using the scale in the [Master View] field.

7. The magnified view of the region selected by the scale is shown in the [Selected View] field.

The vertical chart axis represents the degree and the horizontal axis represents the mapped intensity values.

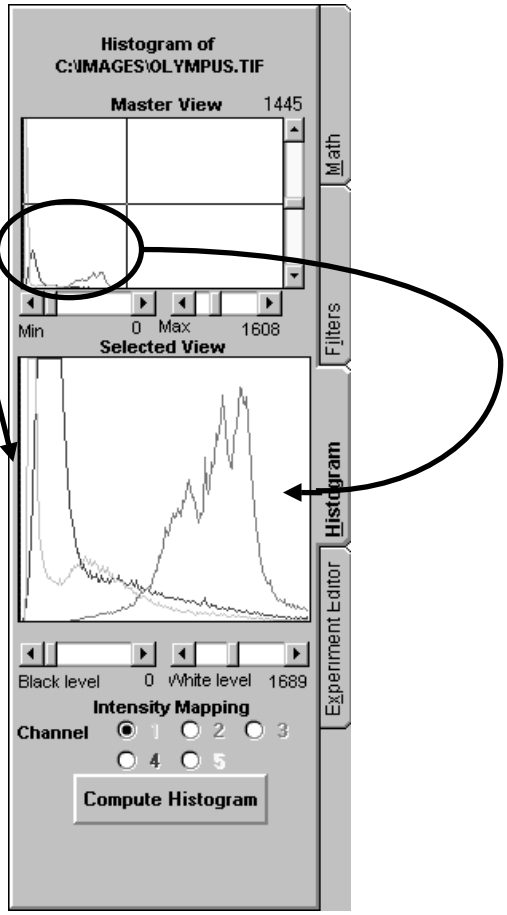


Fig. 2-89 Histogram of the Selected Region

8. When the image was acquired in the multi-channel mode, select the channel(s) to be subjected to LUT intensity mapping (re-assignment) using the [Channel] option buttons.

9. Select the region to be subjected to mapping (re-assignment) using the scale in the [Selected View] field. While moving the scale, confirm the change in contrast in the image window. The intensity values in the selected region are mapped (re-assigned) to intensity values from 0 to 255 and displayed.

2-6-3 Mathematical Operations Between Images

Arithmetic or logical operations can be applied between two different images or between an image and a constant.

2-6-3-1 Image Addition

Addition of an image to an image (constant to an image) is possible as described below.

<Process-Math> button

1. Select <Process-Math> button on tool bar and bring [Math] sub panel to appear.

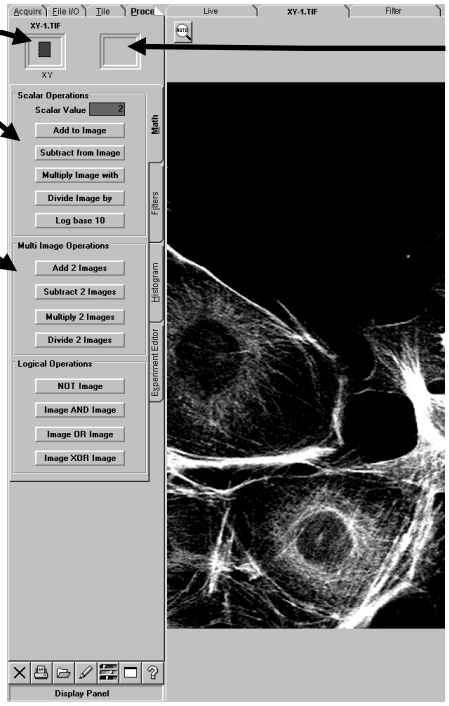
Sets the first image of the operation. The icon of the image is displayed here.

[Scalar Operations] group box

Provides the command buttons for use in operations between image and constant.

[Multi Image Operations] group box

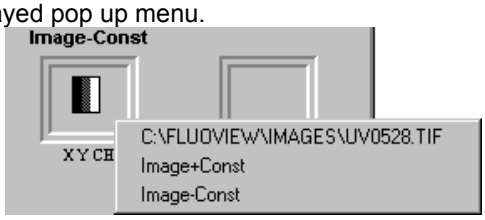
Provides the command buttons for use in operations between two different images.



Sets the second image of the operation. The icon of the image is displayed here. This image needs not be set in operations between image and constant.

Fig. 2-90 [Math] Sub-panel

2. Right click on the frame at the top left of [Math] sub-panel, and select the file name of first image in displayed pop up menu.



TIP

Before the dragging and dropping, the frame at the top left shows the icon of the image file displayed in the image window.

3. Right click on the frame at the top left of [Math] sub-panel, and select the file name of second image in displayed pop up menu.
   (No second file dragging is needed when the image processing is by value.)

4. Enter the constant for use in operation in the [Scalar Value] text box in the [Scalar Operations] group box.
   (This step is required only for operation between an image and a constant.)

<Add 2 Images> button

<Add to Image> button

5. **To add an image to an image:**

   Click the <Add 2 Images> button in the [Multi Image Operations] group box. A new image window showing [Image+Image] in the page tab appears, displaying the image obtained by the addition operation.

   To add a constant to an image:

   Click the <Add to Image> button in the [Scalar Operations] dialog box. A new image window showing [Image+Const] in the page tab appears, displaying the image obtained by the addition operation.

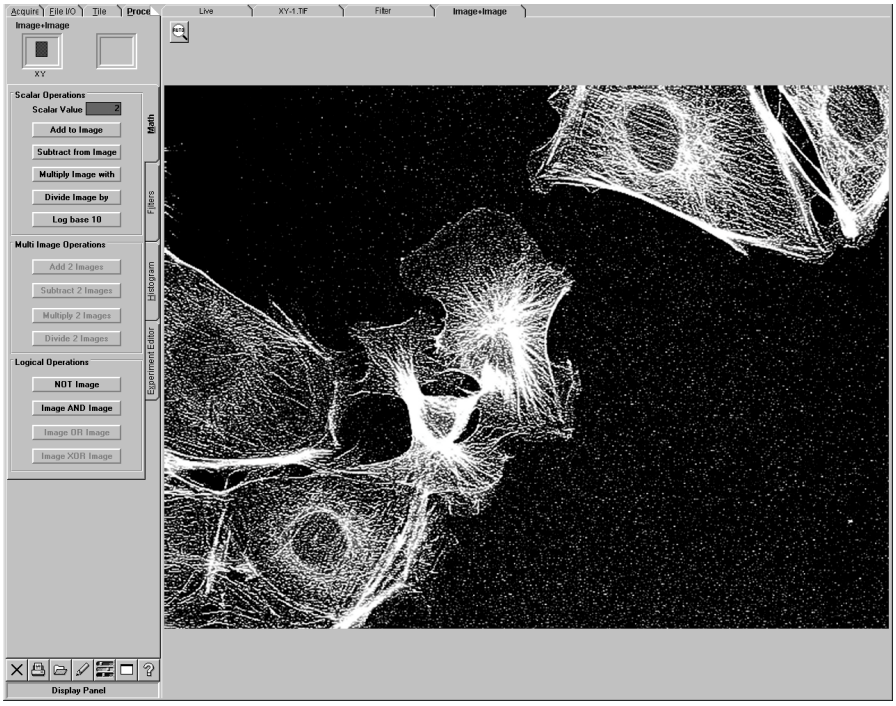


Fig. 2-91 [Image+Image] Panel

2-6-3-2 Image Subtraction

Subtraction of an image from an image (constant from an image) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-6-3-1, “Image Addtion”.

<Subtract 2 Images> button

- **To subtract an image from an image:**

  Click the <Subtract 2 Images> button in the [Multi Image Operations] group box. A new image window showing [Image-Image] in the page tab appears, displaying the image obtained by the subtraction operation.

<Subtract from Image> button

To subtract a constant from an image:

Click the <Subtract from Image> button in the [Scalar Operations] group box. A new image window showing [Image-Const] in the page tab appears, displaying the image obtained by the subtraction operation.

2-6-3-3 Image Multiplication

Multiplication of an image by an image (image by a constant) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-6-3-1, “Image Addition”.

<Multiply 2 Images> buttotn

- **To multiply an image by an image:**

  Click the <Multiply 2 Images> button in the [Multi Image Operations] group box. A new image window showing [Image*Image] in the page tab appears, displaying the image obtained by the multiplication operation.

<Multiply Image with> button

To multiply an image by a constant:

Click the <Multiply Image with> button in the [Scalar Operations] group box. A new image window showing [Image*Const] in the page tab appears, displaying the image obtained by the multiplication operation.

2-6-3-4 Image Division

Division of an image by an image (image by a constant) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-6-3-1, “Image Addition”.

<Divide 2 Images> button

- **To divide an image by an image:**

  Click the <Divide 2 Images> button in the [Multi Image Operations] group box. A new image window showing [Image/Image] in the page tab appears, displaying the image obtained by the division operation.

<Divide Image by> button

To divide an image by a constant:

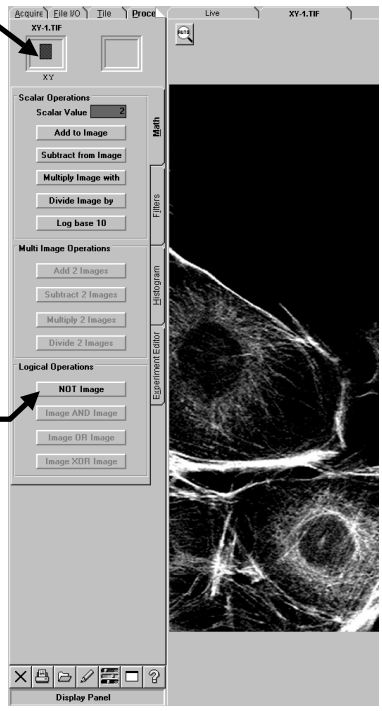
Click the <Divide Image by> button in the [Scalar Operations] group box. A new image window showing [Image/Const] in the page tab appears, displaying the image obtained by the division operation.

2-6-3-5 NOT Image

The NOT operation of an image allows the bright and dark areas of the image to be reversed.

1. Display the [Math] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to operation. The icon of the image is displayed here.



<NOT Image> button
Executes a NOT operation.

Fig. 2-92 [Math] Sub-panel

2. Display the image window of the image to be subjected to NOT operation at the front. The icon of the image is displayed in the frame at the top left of the [Process] panel.

<NOT Image> button

3. Click the <NOT Image> button. A new image window showing [NOT] in the page tab appears, showing the image obtained by the operation.

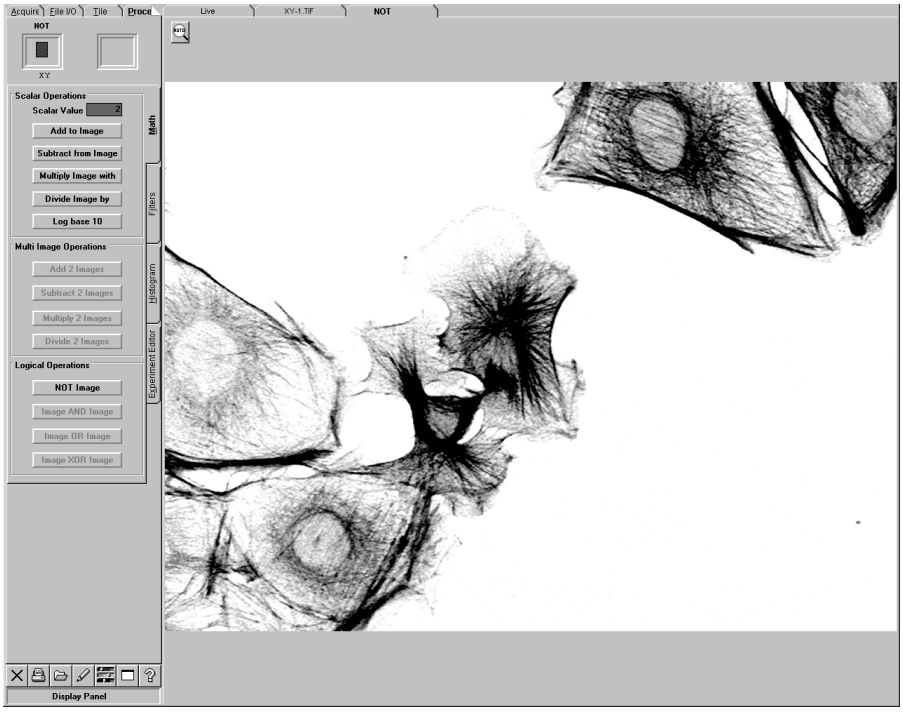


Fig. 2-93 [NOT] Panel

2-6-3-6 Image AND Image

Two different images can be ANDed.

<Process-Math> button

1. Select <Process-Math> button on tool bar and bring [Math] sub panel to appear.

Sets the image to be subjected to operation. The icon of the image is displayed here.

Sets the second image of the operation. The icon of the image is displayed here.

<Image AND Image> button
Executes an AND operation.

<Image OR Image> button
Executes an OR operation.

<Image XOR Image> button
Executes an exclusive OR operation.

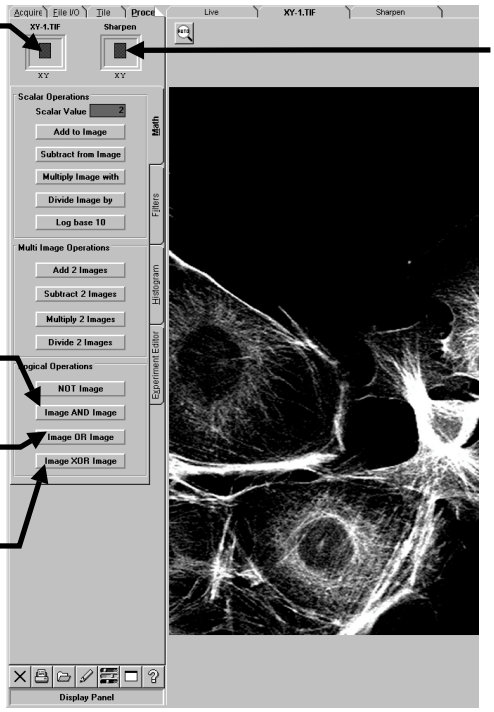


Fig. 2-94 [Math] Sub-panel

2. Right click on the frame at the top left of [Math] sub-panel, and select the file name of first image in displayed pop up menu.

TIP

Before the dragging and dropping, the frame at the top left shows the icon of the image file displayed in the image window.

3. Right click on the frame at the top left of [Math] sub-panel, and select the file name of second image in displayed pop up menu.

<Image AND Image> button

4. Click the <Image AND Image> button. A new image window showing [Image AND Image] in the page tab appears, showing the image obtained by the operation.

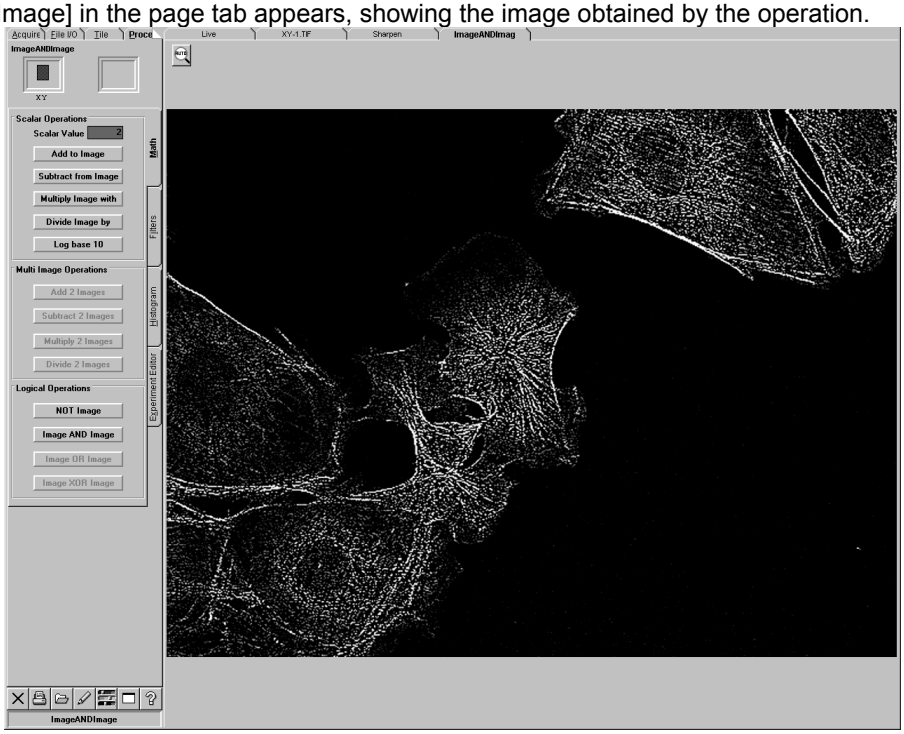


Fig. 2-95 [Image AND Image] Panel

2-6-3-7 Image OR Image

Two different images can be ORed.

The operation method is identical to the AND operation between two different images except for the following point. See section 2-6-3-6, “Image AND Image”.

<Image OR Image> button

- Click the <Image OR Image> button. A new image window showing [Image OR Image] in the page tab appears, showing the image obtained by the operation.

2-6-3-8 Image XOR Image

Two different images can be XORed.

The operation method is identical to the AND operation between two different images except for the following point. See section 2-6-3-6, “Image AND Image”.

<Image XOR Image> button

- Click the <Image XOR Image> button. A new image window showing [Image XOR Image] in the page tab appears, showing the image obtained by the operation.

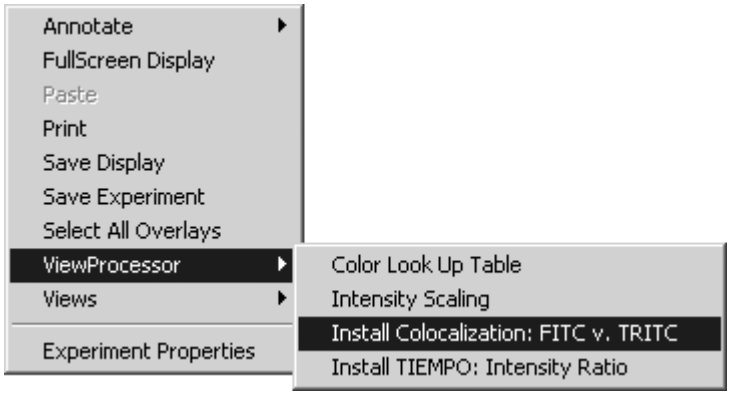
2-6-4 Brightness overlap level between 2 channels (Colocalization)

This function enables to observe overlap intensity of two channel of image.

1. Acquires or opens images which more than two channels, and displays the images on image window.

2. On the image window, displays and overlaps 2 channel image that to be observed. See section 2-4-4-2 for overlapping the image display.

3. On the overlapped images, click right-mouse button to show the following pop up menu.



4. Select [Install Colocalization: ****.v ****] of [ViewProcessor]. Then [Colocalization Processor] appears.
   [****] shows the dyeing method or its number of channel.

There are the following 4 Annotation Mode in [Colocalization Processor] dialog box.

- None Mode
- Thresholds Mode
- Min-Max Bounds Mode
- Regions Mode

2-6-4-1 Annotation Mode

1 None Mode

None Mode is the default of [Colocalization Processor] dialog box. The mode can be changed by option button in [Annotation Mode] Group Box. The items described for this mode can be used in other modes.

[Data Selection] Group Box

<Disabled> Option Button

Graph and Text box will disappear from

<Current slice> Option Button

This button executes colocaliztion for images displayed on image window.

[Histogram] Graph

It acquires brightness value of 1st channel selected on horizontal axis and brightness of value of other channel on vertical axis and displays brightness distribution. The point with the value which less than [LUT min] text box is colored with blue, and the point more than [LUT max] value is colored with in red; and other point will be colored by gray scale.

[Summary] Text Box

Summary : Dye method of vertical and horizontal axis or channel number
Mode : Option button selected at [Data Selection] group box
Samples processed : Total numbers of pixels on whole image to be processed
Binning : [Bin Width] Setting condition of text box
Max. samples in any bin : Max. value in plot data on graph

[Annotation Mode] Group Box

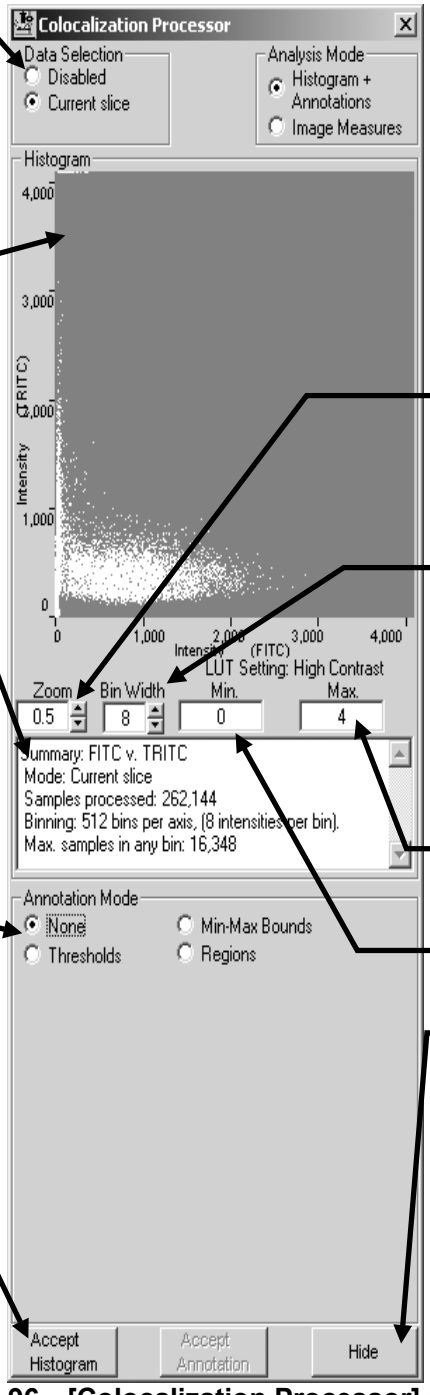
Select either [None], [Threshold], [Min-Max Bounds] or [Regions] option button. In case of [Threshold], [Min-Max Bounds] or [Regions] are selected, text box appears in [Annotation] Group Box. [Colocalization Processor] is displayed, [None] is selected.

<Accept Histogram> Button

[Histogram] graph appears in image window.

The graph can be handled same with the acquired image.

Some measuring results indicated may have the computation error while the software process.



[Zoom] Text Box

It sets display magnification of [Histogram] graph.

[Bin Width] Text Box

It sets resolution of [Histogram] graph. For example, if the value is 16, it displays brightness in 16 gray-scale as one plot.

[LUT Max] Text Box

Double-click inside text box so that the display can alternately be changed in two methods.
1. All data uniform display
2. Binary display of data presence

[LUT Min] Text Box

<Hide> Button

It hides [Colocalization Processor] dialog box.

The [Colocalization Processor] dialog box appears again by pressing <Colocalization Processor> button in image window.

Fig. 2-96 [Colocalization Processor] Dialog Box In case of Annotation Mode is [None].

2 Thresholds Mode

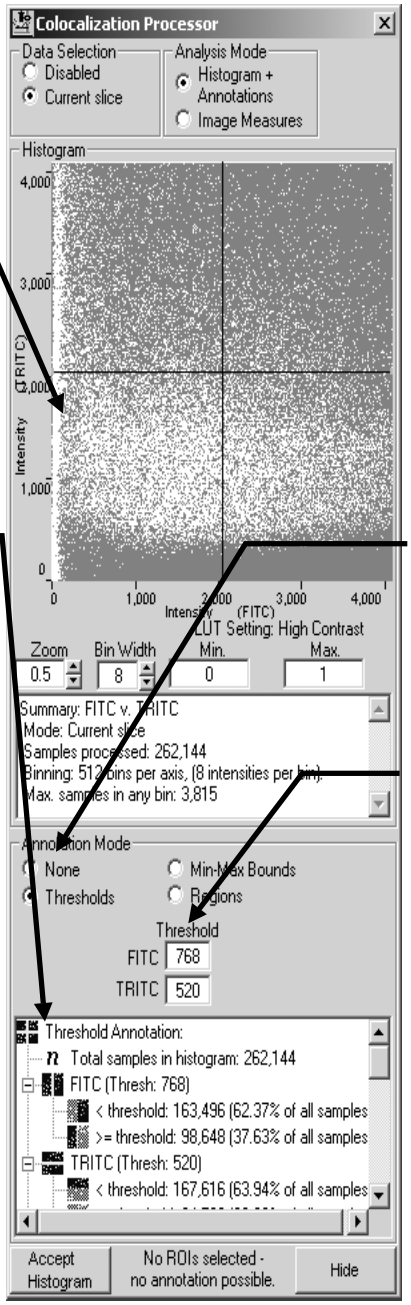
Specifies Threshold level on [Histogram] graph, then measures by use of the threshold level.

[Histogram] Graph

Threshold line vertical and horizontal red line) appears. The value can be adjust by dragging those lines.

[Annotation] Text Box

For details, refer to the explanation that appears in next page.



[Annotation mode] Group Box

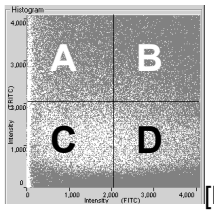
When <Thresholds> option button is selected, this dialog box appears.

[Threshold] Text Box

Threshold values of X and Y axis are displayed on [Histogram] graph. It can also be input from the keyboard
The value entered will be automatically changed to the value that can be processed in software.

Fig. 2-97 [Colocalization Processor] Dialog Box

In case of Annotation Mode is [Threshold].



[Histogram] graph

Explanation of [Annotation] Text Box

Display in Text Box	Explanation
Threshold Annotation	Thresholds Mode
Total samples in histogram:	Total numbers of image pixels to be processed
(Dye-name1)[Thresh:]	Name of Dye method. Threshold value in X-axis.
<threshold:	Total numbers of image pixels below the threshold value in X-axis ((A+C)/(A+B+C+D))x100 [%]
>=threshold:	Total numbers of image pixels above the threshold value in X-axis((B+D)/(A+B+C+D))x100 [%]
(Dye-name2)[Thresh:]	Name of Dye method. Threshold value in Y-axis
<threshold:	Total numbers of image pixels below the threshold value in Y-axis ((C+D)/(A+B+C+D))x100 [%]
>=threshold:	Total numbers of image pixels above the threshold value in Y-axis ((A+B)/(A+B+C+D))x100 [%]
Upper-left	Upper left portion of [histogram] graph
Samples	Total numbers of image pixels contained in upper left portion
[of (Dye-name1) < threshold]	(A/(A+C))x100 [%]
[of (Dye-name2) >= threshold]	(A/(A+B))x100 [%]
Of all samples	(A/(A+B+C+D))x100 [%]
Upper-right	Upper right portion of [histogram] graph
Samples	Total numbers of image pixels contained in upper right portion
[of (Dye-name1) >= threshold]	(B/(B+D))x100 [%]
[of (Dye-name2) >= threshold]	(B/(A+B))x100 [%]
Of all samples	(B/(A+B+C+D))x100 [%]
Lower-left	Lower left portion of [histogram] graph
Samples	Total numbers of image pixels contained in lower left portion
[of (Dye-name1) < threshold]	(C/(A+C))x100 [%]
[of (Dye-name2) < threshold]	(C/(C+D))x100 [%]
Of all samples	(C/(A+B+C+D))x100 [%]
Lower-right	Lower right portion of [histogram] graph
Samples	Total numbers of image pixels contained in lower right portion
[of (Dye-name1) >= threshold]	(D/(B+D))x100 [%]
[of (Dye-name2) < threshold]	(D/(C+D))x100 [%]
Of all samples	(D/(A+B+C+D))x100 [%]

3 Min-Max Bounds Mode

Specifies the rectangular on [Histogram] graph, and measures colocalization by use of the rectangular.

In addition, distribution level of brightness inside rectangular is displayed on image window. Color is the same as color of rectangular selected.

[Histogram] Graph

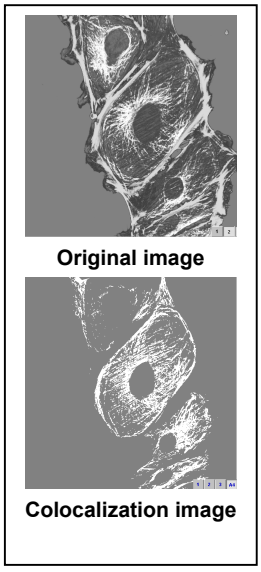
Rectangle is displayed in graph. The rectangular can be positioned by dragging.

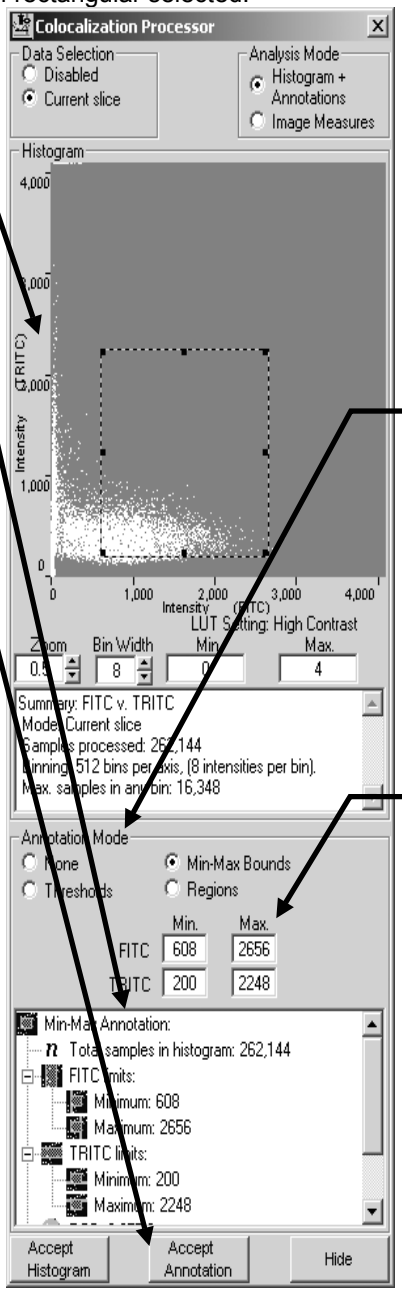
[Annotation] Text Box

For details, refer to the explanation that appears in next page.

<Accept Annotation> button

It extracts brightness only of image designated in rectangular on [Histogram] graph and creates new image window and displays the image.





[Annotation mode] Group Box

When < Min-Max Bounds > option button is selected, this dialog will appear.

[Min.] Text Box

[Max.] Text Box

Max. and Min. Positions of rectangle on [Histogram] graph will be displayed. You can input the values directly and change the values.

The value entered will be automatically changed to the value that can be processed in software.

If the same value is entered in both [Min.] and [Max.] text boxes of the same dyeing method, it does not form a rectangle and it is not possible to execute the measurement.

Fig. 2-98 [Colocalization Processor] Dialog Box

In case of Annotation Mode is [Min-Max Bounds].

Explanation of [Annotation] Text Box

Display in Text Box	**Explanation**
Min-Max Annotation	Min-Max Bounds Mode
Total samples in histogram	Total numbers of image pixels to be processed
(Dye-name1) limits:	X-axis range of rectangular
Minimum:	Minimum value of X-axis rectangular
Maximum:	Maximum value of X-axis rectangular
(Dye-name2) limits:	Y-axis range of rectangular
Minimum:	Minimum value of Y-axis rectangular
Maximum:	Maximum value of Y-axis rectangular
RGB:	Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
Samples outside region:	Total number of image pixels outside rectangular and ratio to whole region

4 Regions Mode

Specifies the arbitrary region in [Histogram], and measures colocalization within the region. In addition, distribution level of brightness in draw pictorial figure is displayed on image window. Color is the same as color of draw pictorial figure selected.

[Histogram] Graph

You can draw a pictorial figure on [Histogram], using graphic button located at lower right side of dialog box.
See 2-12 Entering Comment in Image for methods to delete graphic, to select plural numbers of graphics and to change color of graphic frame.

[Annotation mode] Group Box

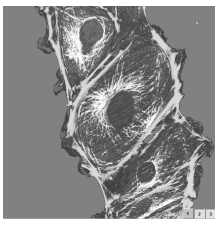
When < Regions > option button is selected, this dialog box will appear.

[Annotation] Text Box

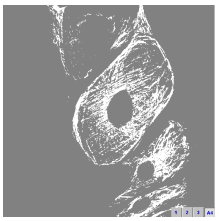
For details, refer to the explanation that appears in next page.

<Accept Annotation> button

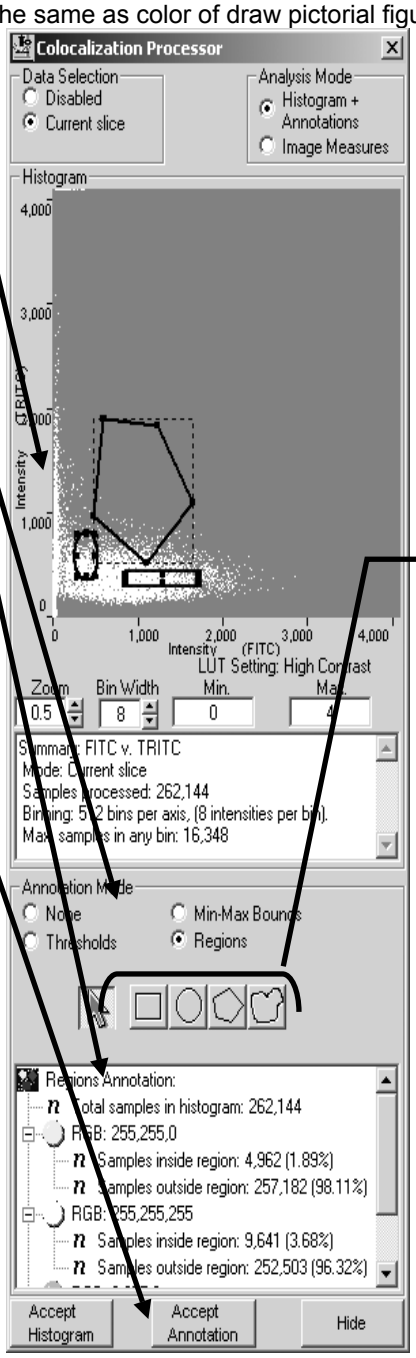
It extracts brightness only of image designated in rectangular on [Histogram] graph and creates new image window and displays the image.



Original image



Colocalization image



<Rectangular> Button

Rectangular draw: On graph, drag diagonally from upper left to lower right to draw rectangular region.

<Circle> Button

Circle/Oval draw: Drag diagonally rectangle that circumscribes region of the circle to be drawn on the image.

<Poly region> Button

Polygon draw: Click each peak to draw a polygon. When the last peak is clicked, the peak clicked at first time will be connected with the last peak.

<Free Region> Button

Free draw: Specify region by dragging on image. Releasing the mouse button at the end of drag, end and start positions are connected.

Fig. 2-99 [Colocalization Processor] Dialog Box
In case of Annotation Mode is [Regions]

Explanation of [Annotation] Text Box (In case that 3 pictorial figures exist. Items (1) will be increased according to number of pictorial figures.).

	Display in Text Box	Explanation
	Regions Annotation	Regions Mode
	Total samples in histogram	Total numbers of image pixels to be processed
(1)	RGB:	1st pictorial figure. Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
(1)	Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
(1)	Samples outside region:	Total numbers of image pixels outside rectangular and ratio to whole region
	RGB:	2nd pictorial figure. Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
	Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
	Samples outside region:	Total numbers of image pixels outside rectangular and ratio to whole region
	RGB:	3rd pictorial figure. Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
	Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
	Samples outside region:	Total numbers of image pixels outside rectangular and ratio to whole region

2-6-4-2 Colocalization for series image data set

Colocalization is also available for series image data set, i.e XYZ image data set.

[Data Selection] Group Box

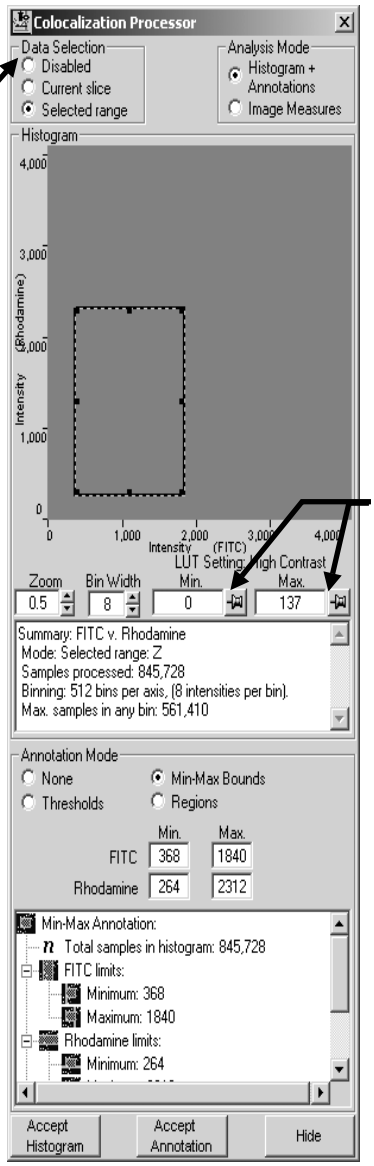
<Current slice> Option Button

The cocalization is processed for the image in a series data set which is displayed in image window.

<Selected range> Option Button

This button appears when series data set is in use.

The colocalization is processed on to the all image of the data set.



<Draw> Button

<Draw> button appears when image data set in use.

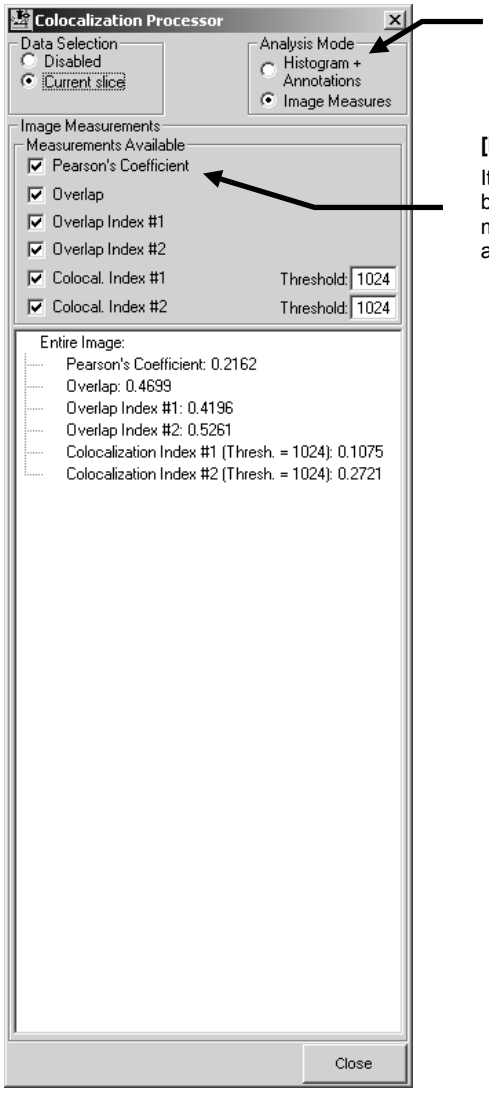
When <Current Slice> option button is selected at [Data Selection] group box, and <Draw> buttons at right side of text boxes – [LUT Max] and [LUT Min] are depressed, the values in text boxes will not change even if Current Slice image displayed on image window is changed. In case that <Draw> buttons are popped out, the values will change with the Current Slice change.

Fig. 2-100 [Colocalization Processor] Dialog Box

Colocalization for series image data set

2-6-4-3 Image measurement

When option button of [Image Measures] of [Analysis Mode] Group Box is selected, the dialog as shown below appears.



[Analysis Mode] Group Box

[Image Measurements] Group Box

It displays results of measurement in text box below. For details of measurement method, refer to the explanation that appears in next page.

Fig. 2-101 [Colocalization Processor] Dialog box
In case that Analysis Mode is Image Measures

· Pearson's Coefficient :

In case that the value to be acquired is defined as r ,

$$r = \frac{\sum_i (F_{1,i} - \overline{F_{1,i}}) \cdot (F_{2,i} - \overline{F_{2,i}})}{\sqrt{\sum_i (F_{1,i} - F_{1,i})^2 \cdot \sum_i (F_{2,i} - \overline{F_{2,i}})^2}}$$

However, legends used for image measurement are as follows:
$F_{1,i}$: Brightness of wavelength λ_1 at i -th pixel, $F_{2,i}$: Brightness of wavelength λ_2 at i -th pixel

$\overline{F_{1,i}} = \frac{1}{N}\sum_i F_{1,i}$: Average brightness of wavelength λ_1 , $\overline{F_{2,i}} = \frac{1}{N}\sum_i F_{2,i}$: Average brightness of wavelength λ_2

· Overlap :

In case that the value to be acquired is defined as r ,

$$r = \frac{\sum_i F_{1,i} \cdot F_{2,i}}{\sqrt{\sum_i {F_{1,i}}^2} \cdot \sqrt{\sum_i {F_{2,i}}^2}}$$

· Overlap Index #1 :

In case that the value to be acquired is defined as k_1 ,

$$k_1 = \frac{\sum_i F_{1,i} \cdot F_{2,i}}{\sum_i F_{1,i}^2}$$

· Overlap Index #2 :

In case that the value to be acquired is defined as k_2 ,

$$k_2 = \frac{\sum_i F_{1,i} \cdot F_{2,i}}{\sum_i F_{2,i}^2}$$

· Colocalization Index #1 :

In case that the value to be acquired is defined as m_1 ,

$$m_1 = \frac{\sum_{i \in A} F_{1,i}}{\sum_i F_{1,i}}$$

However, $i \in A$ should be i -th pixel that belongs to aggregation A that is greater than threshold value.

· Colocalization Index #2 :

In case that the value to be acquired is defined as m_2,

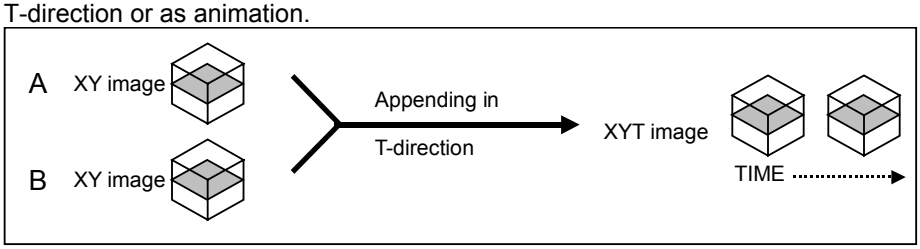
$$m_2 = \frac{\sum_{i \in A} F_{2,i}}{\sum_{i} F_{2,i}}$$

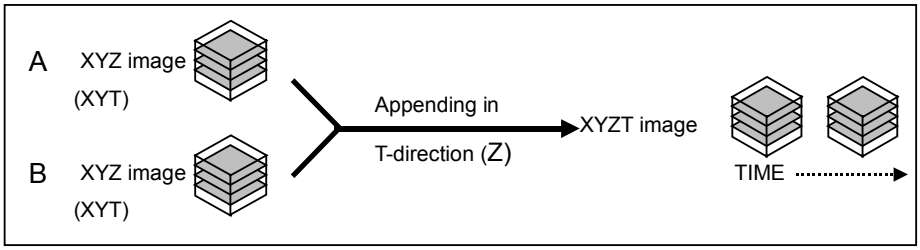
However, $i \in A$ should be i-th pixel that belongs to aggregation A that is greater than threhold value.

2-6-5 Appending image (Append)

2-6-5-1 Appending two images

Two images (A and B) acquired in XY or XYZ observation can be appended along the Z- or T-direction or as animation.





NOTE

The image files used in appending are subjected to the following restrictions.

- **The sizes of the two image files should be identical.**
- **The numbers of channels of the two image files should be identical.**
- **The T- or Z-observation conditions (interval times, acquisition times, number of acquisitions, number of steps, etc.) of the two image files should be identical.**
- **The combination of the two image files should be a combination with which appending is permitted (see the table on page 2-273).**

Two XY images can be appended in the T-direction with the following procedure.

1. Open the two image files (A and B) for appending.

2. Display the image window of the first image (A) at the front.

3. Display the [Experiment Editor] sub-panel of the [Process] panel at the front.

Fig. 2-102 [Experiment Editor] Sub-panel

<Experiment List> button

4. In the toolbar at the bottom of the [Process] panel, click the <Experiment List> button. The [Experiments in Memory] dialog box appears as shown below.

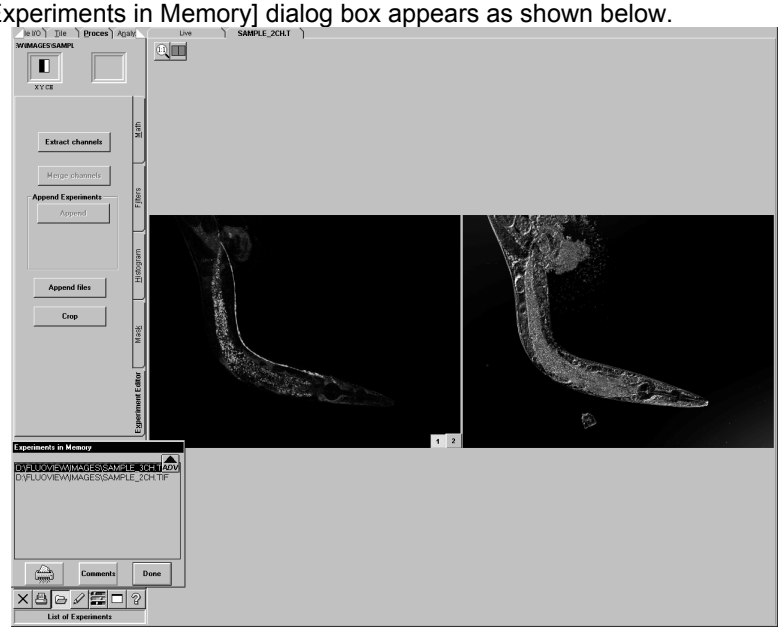
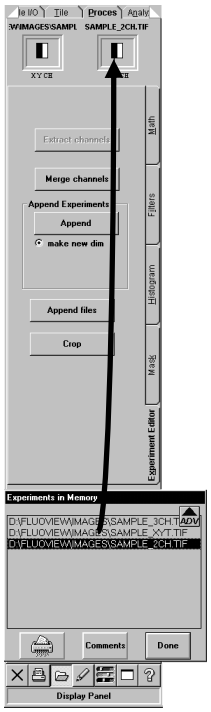


Fig. 2-103 [Experiments in Memory] Dialog Box

TIP

The icon of the image file being displayed at the front of the image window (A) is shown in the frame at the top left of the [Process] panel.



5. In the [Experiments in Memory] dialog box, select the file name of the second image (B) and drag it to the frame at the top right of the [Process] panel. The icon of the second image will be displayed in the frame at the top right of the [Process] panel.

TIP

The mouse pointer turns into the image icon during dragging.

6. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

7. Click the <Append> button in the [Experiment Editor] subpanel.

The [Append on New Dimension] dialog box appears as shown below.

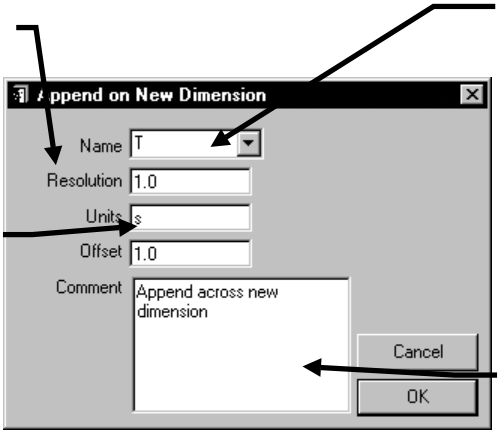
[Resolution] text box

Specify the interval time (in seconds) when "T" is selected in the [Name] drop-down list.

Specify the number of steps when "Z" is selected in the [Name] drop-down list.

[Units] text box

Specify the unit which utilize to [Resolution].



[Name] drop-down list

Select the type of the image to be created by appending.

T: Time-lapse image (T-direction)

Z: Z-series image (Z-direction)

AN: Animation image

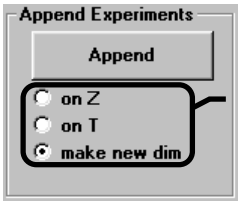
St: 3D image or stereo image to be viewed through color (red/green) eyeglasses

[Comment] text box

Enter the comment for the created image.

TIP

Depending on the type of the image to be created by appending, various option buttons appears under the <Append> button in the [Experiment Editor] sub-panel.



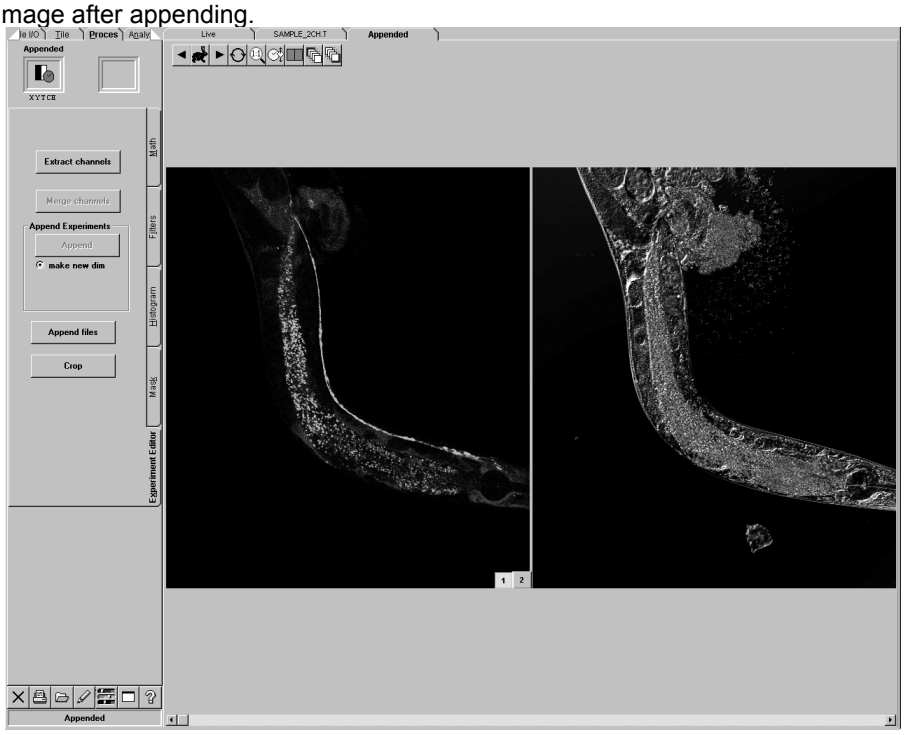
Option buttons depending on the image mode.

· make new dim .. Create an image of new observation mode. (Select the type in the [Append on New Dimension] dialog box.)

· on Z ..... Append along the Z-direction.

· on T ..... Append along the T-direction.

8. A new image window having [Append] as the window title is created, showing the image after appending.



TIP

For appending more than 3 images at a time, see section 2-6-5-2.

2-6-5-2 Appending image from several image data set

An image data set can be made from appending several image data set.

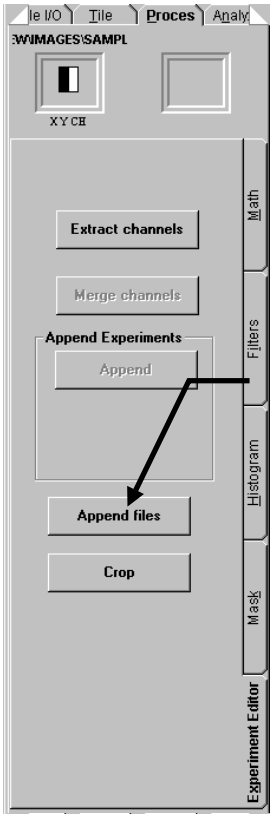
NOTE

There are the following restrictions when you append image files.

·Two image files must be the same image size.

·Two image files must be the same channel number.

·Two image files must be a combination that can be appended (see 2-253 page).



1. Click <Append files> button on [Experiment Editor] sub panel of [Process] panel.

<Append files> button

2. [Append on New dimension] dialog box appears as shown below.

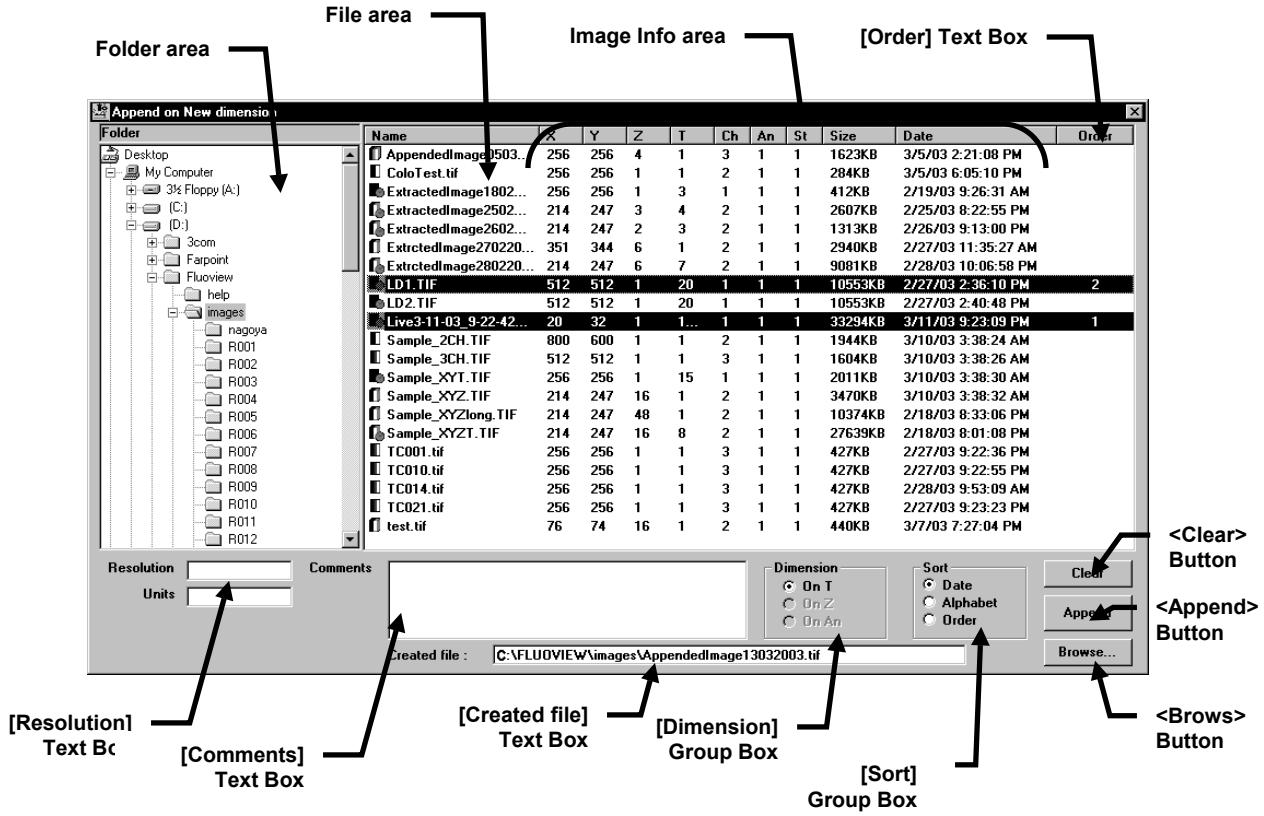


Fig. 2-104 [Append New dimension] Dialog Box

3. Select file folder on Folder area. Then, MultiTif image file name appears in File area.

4. Select image files to be appended.

TIP

·Image files can be selected by dragging file name in File area.

·Several image files can assign by using Click and Shift-key + Click in File area.

·Several image files can also assign by Click with Ctrl-key.

Order of the file selection appears in [Order] text box area.

5. Select Series data set type that will be created in [Dimension] Group Box.

TIP

The option buttons work as the followings.

·on T..... Appends file in T direction.

·on Z..... Appends file in Z direction.

·on An ..... Appends file as animation.

TIP

Time information of each file stays as its original, in case of T direction appending.

Resolution information will be taken over when file of different Z Resolution information is appended, in case of Z direction appending.

6. Select the order for appending image data with [Sort] Group Box.

TIP

The option buttons work as the followings.

·Date ..... Appends in order of date.

·Alphabet..... Appends in alphabetical order.

·Order..... Appends in order which is specified at[Order].

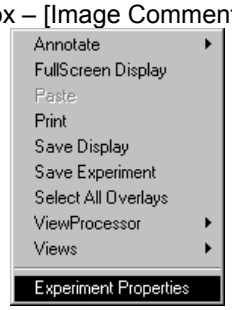
7. Enter comment into [Comments] dialog box.

TIP

Comment entered can be reviewed as follows.

1.Display appended image on image window.

2.Do mouse-right-click over the image and select [Experiment Properties] from pop up menu. Dialog box – [Image Comments] will appear.



3. Select tab – [Comments] on [Image Comments] dialog box so that commept will be displayed in the text box.

8. Enter folder and file name into [Create file] text box.
   By pressing <Browse…> button, folder appears for easy folder selection.

9. Click <Append> button, then new appended image created and stored.

TIP

Pressing <Clear> button clears the file assignment.

TIP

[Resolution] text box works as follows.

·In case of T in [Dimension] group box:

All images are time-series: Input not possible.

Image is not time-series: The setting is utilized for the time stamp interval that starts with 0 sec.

·In case of Z in [Dimension] group box

The setting is utilized for the Z step interval that starts with 0 um.

·In case of on An in [Dimension] group box:

The setting is utilized for pixel intensity step that starts with intensity 0.

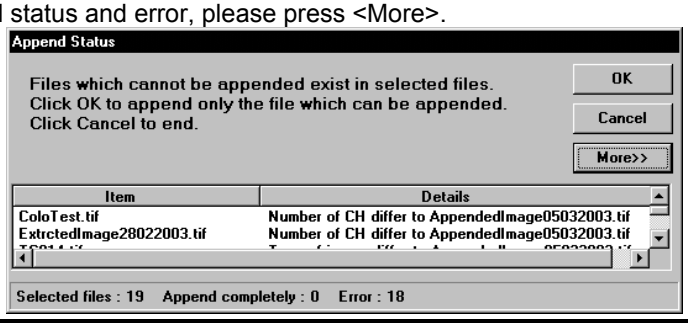
TIP

Depending upon image size and number of slices to be appended, it may take a few minutes or several tens of minutes to process for appending. Progress bar will appear to indicate the progress during appending.

One Point!

[Append Status] dialog box appears when images can not appended. Pressing <OK>, the image is appended only for those are possible to be appended.

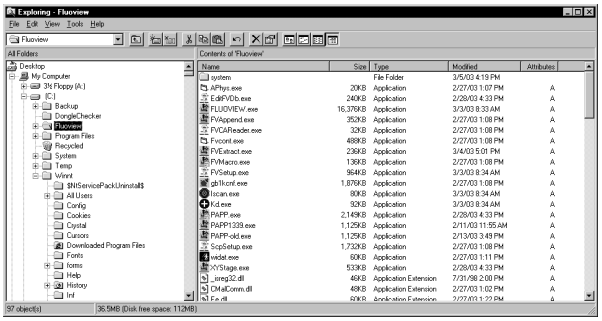
To see detailed status and error, please press <More>.


Append Status
Files which cannot be appended exist in selected files.
Click OK to append only the file which can be appended.
Click Cancel to end.
OK
Cancel
More>>
Item
Details
ColoTest.tif
Number of CH differ to AppendedImage05032003.tif
ExtrctedImage28022003.tif
Number of CH differ to AppendedImage05032003.tif
Selected files : 19 Append completely : 0 Error : 18

One Point!

Plural numbers of images can be appended without starting FLUOVIEW software.

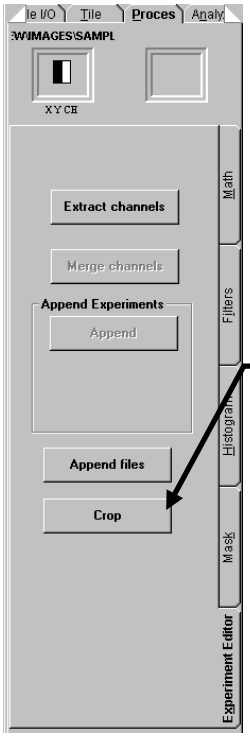
1. Select <Start> button at bottom of Windows screen.
2. In case of Windows XP, select [All Programs] – [Accessories] - [Windows Explorer] command from the [start] menu. In case of Windows 2000, select [Accessories] - [Windows Explorer] command from the [start] menu. As shown below, Windows XP or Windows 2000 Explorer will appear.



3. Double click [FVAppend.exe] in [FLUOVIEW] folder at [C:] drive. Dialog box - [Append on New dimension] will appear.
4. In case that the same operation as the procedures described in this manual is done, the image will be appended and a new file will be created.

2-6-6 Extract image (Crop)

New image data with selected slice image from an image data set can be made by this function.



The following is an example for Image extraction from an XYZT image data set.

1. Display the image to be extract in image window.

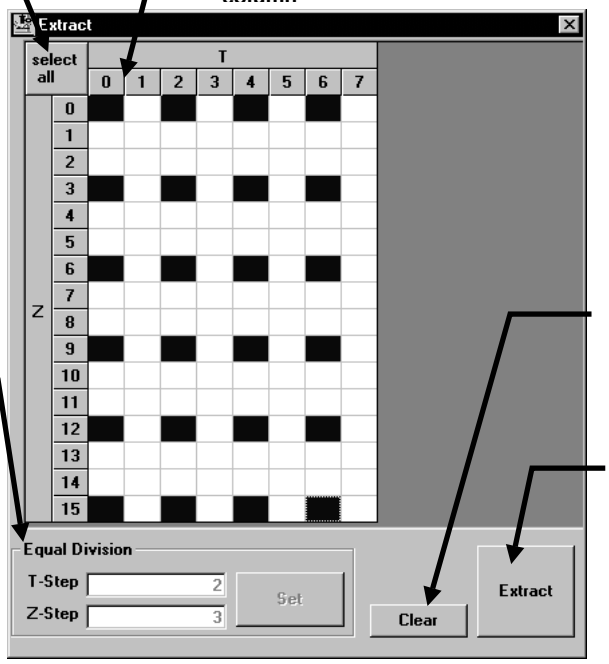
2. Select [Experiment Editor] sub panel in [Process] panel.

<Crop> button

3. By clicking <Crop> button. [Extract] dialog box appears.

<select all> button

[No. Display] column



<Clear> Button

<Extract> Button

[Equal Division] Group Box

- **[T-Step] Text Box**
- **[Z-Step] Text Box**
- **<Set> Button**

Fig. 2-105 [Extract] Dialog box

4. Select image slices by pressing on [Extract] dialog box.

TIP

The following method of slice selection is also available.

·All of image slices can be select by pressing <select all> button.

·All of row or column slices can be selected by pressing [No. Display].

·Image slices with a certain time interval and/or Z step can be selected by pressing <Set> after set [T-Step] and/or [Z-Step] in [Equal Division].

·Image slices with a certain rectangular area on [Extract] panel can be selected by dragging from top-left to bottom-right.

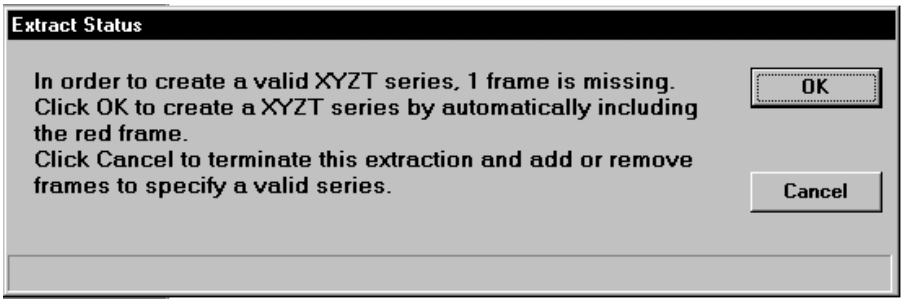
TIP

The slice assignment cleared by clicking <clear> button in [Extract] dialog box.

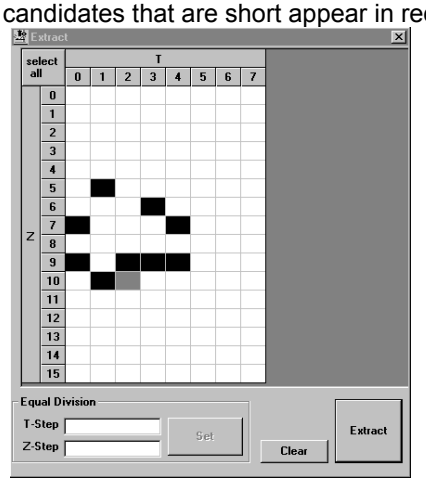
5. Click <Extract> button the extracted image appears in image window with named [Crop].

One Point!

When arbitrary portion of XYT image is extracted and plural numbers of [No. Display] columns at T columns are selected, the dialog box as shown below will appear in case that number of units at Z portion of [No. Display] on all T columns are not the same number of units.


Extract Status
In order to create a valid XYZT series, 1 frame is missing.
Click OK to create a XYZT series by automatically including the red frame.
Click Cancel to terminate this extraction and add or remove frames to specify a valid series.
OK
Cancel

In [Extract] dialog box, grid candidates that are short appear in red.



Selection of grid candidates that are short is done as follows.

1. Verification will be done whether or not the same numbers of Z elements are selected against all grids in T direction.
2. When there are grids in which Z elements are short, the selection of Z element position equal to Z element that corresponds to previous T element will automatically be done.

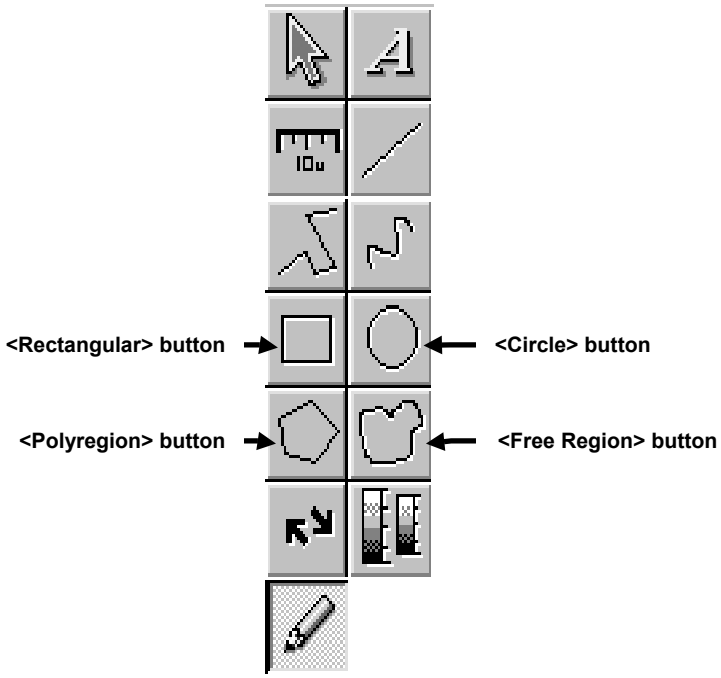
When <OK> button of [Extract Status] dialog box is clicked, the extraction for grids including the grids displayed in red will be done . When <Cancel> button is clicked, extraction will be aborted.

One Point!

It is possible to cut out the image and extract it by specifying arbitrary region. Image cut out is done as follows.

<Annotate> button

1. Select [Annotate] button in tool bar.



2. Select either <Rectangular>, <Circle>, <Polyregion> or <Free Region> to assign clipping area.

   When option other than <Rectangular> is selected, the cut out (clipping) will be done against rectangle that circumscribes the specified profile.

3. Follow procedures subsequent to 3 of section 2-6-6-1 and extract the image. The image will be cut out and extracted and new image will be displayed.

One Point!

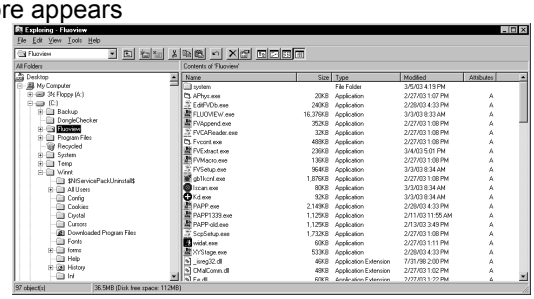
Slice extract function works even if FLUOVIEW software is not running. However, clipping function works only with FLUOVIEW software.

1.Press Windows <Start> button.

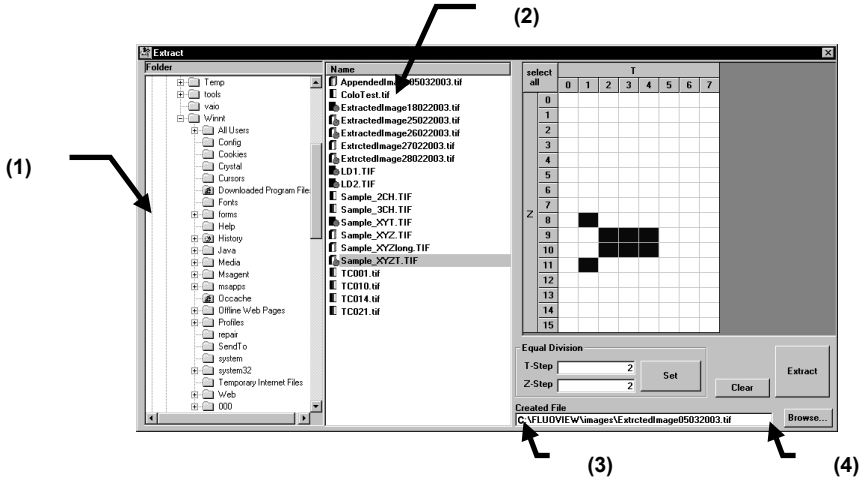
2.In case of Windows XP, select [All Programs] – [Accessories] - [Windows Explorer] command from the [start] menu.

In case of Windows 2000, select [Accessories] – [Windows Explorer] command from the [start] menu.

Then, Windows Explore appears



3.Double-click FVExtract.exe in Fluoview of C:Drive..



4. Assign folder (1) Folder area to be image data stored.

5.Select image data in (2) File area to be extracted.

6.File and its folder name appears in <Created file> text box, after pressing <Browse…> and set file and folder name to be stored.

7.Image is extracted and new image data file is created by following procedures this section.

Combinations of images with which appending are possible

A \ B	XY	XYZ	XYT	XYZT	XZ	XZT	XT	Pt-Pt	XY-AN
XY	XYZ XYT XY-AN	XYZ	XYT						XY-AN
XYZ	XYZ	XYZ XYZT		XYZT					
XYT	XYT		XYT						
XYZT		XYZT		XYZT					
XZ					XZT	XZT			
XZT					XZT	XZT			
XT							XT-AN		
Pt-Pt								Pt-Pt-AN	
XY-AN	XY-AN								XY-AN

Pt..... Point-scanned image

AN ..... Animation image

2-7 Image Analysis

Images can be analyzed using the [Analyze] panel. Display the [Analyze] panel at the front.

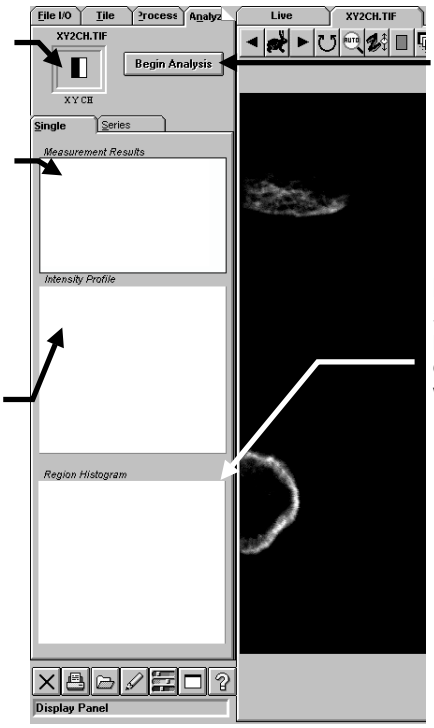
Displays the icon of the image being displayed (image to be subjected to analysis).

[Measurement Results] box

Shows the measurement data of the specified line or region.

[Intensity Profile] box

Shows the intensity profile chart of the specified line or region. When a line is specified, the line profile is displayed, and double-clicking this field displays the [Enhanced Profile Plot] window. When a region is specified, the bird's eye view is displayed, and double-clicking this field displays the [Intensity Map] window.



<Begin Analysis> button

Starts analysis.

[Region Histogram] box

Shows the histogram of the specified line or region. Double-clicking this field displays the [Enhanced Histogram Plot] window.

Fig. 2-106 [Analyze] Panel

2-7-1 Checking the Intensity of a Specific Part

2-7-1-1 Intensity Values on a Line (Line Profile)

The intensity values on a line in an image can be displayed graphically.

1. Display the [Single] sub-panel at the front.
2. Display the image window of the image to be subjected to the intensity checking at the front.
3. Click the <Annotate> button in the toolbar. A list of buttons appears as shown below.

<Annotate> button

4. From the displayed buttons, click the <Line> button, <Poly Line> button or <Free Line> button.
5. Specify the straight line, polygonal line or free line on the image in the image window. They can be specified as described below.

<Line> button

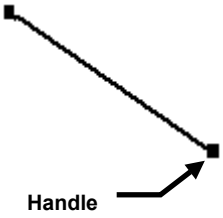
- To specify a straight line:
  On the image, place the mouse pointer on the point you want to start the straight line and drag until the point you want to end it.

<Poly Line> button

- To specify a polygonal line:
  On the image, click the points corresponding to the start point, peak points and end point of the desired polygonal line, then click the right button of the mouse to set the specification.

<Free Line>button

- To specify a free line:
  On the image, drag the mouse pointer along the line to be checked.
  The line is displayed on the image together with the handles on it. The intensity profile can be displayed while the handles are displayed.



NOTE

When the moues is clicked in other place than on the specified line, the handles on the line disappear. The intensity profile cannot be displayed when the handles are not displayed.

TIP

The checked line can be moved, deleted or changed of size or color.
This is possible with the same method as entering comment in the image.
For details, see sections 2-12-6, 2-12-7, 2-12-8 and 2-12-9 in section 2-12, “Entering Comment in Image”.

<Annotate> button

6. Click the <Annotate> button so that the list of buttons disappears.
7. Click the <Begin Analysis> button. The intensity profile of the specified line will be displayed in the [Intensity Profile] box of the [Analyze] panel.

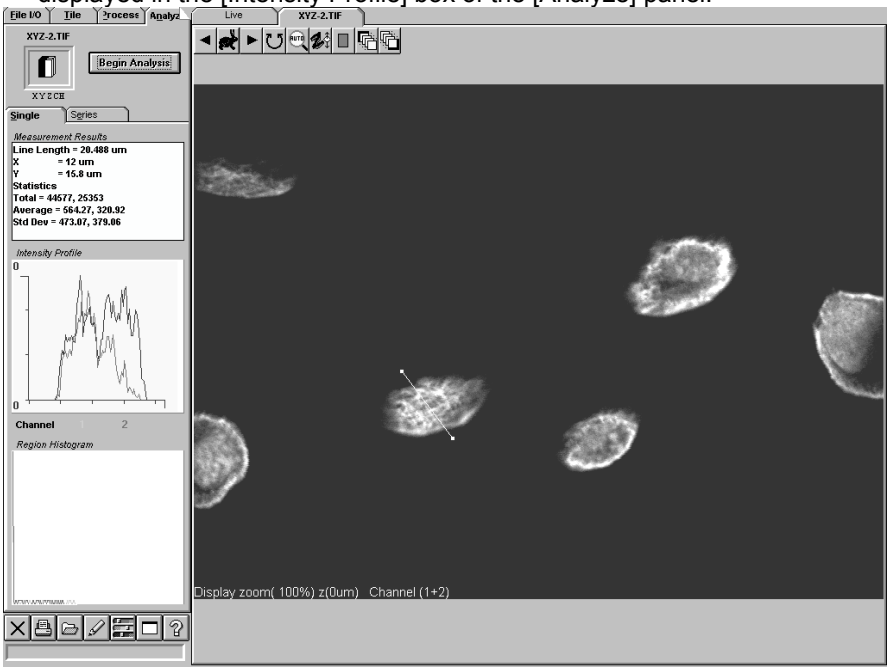


Fig. 2-107 Panel After Analysis (Line Specification)

8. Double-click the [Intensity Profile] button. The [Enhanced Profile] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, "Changing the Chart Display Method" for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

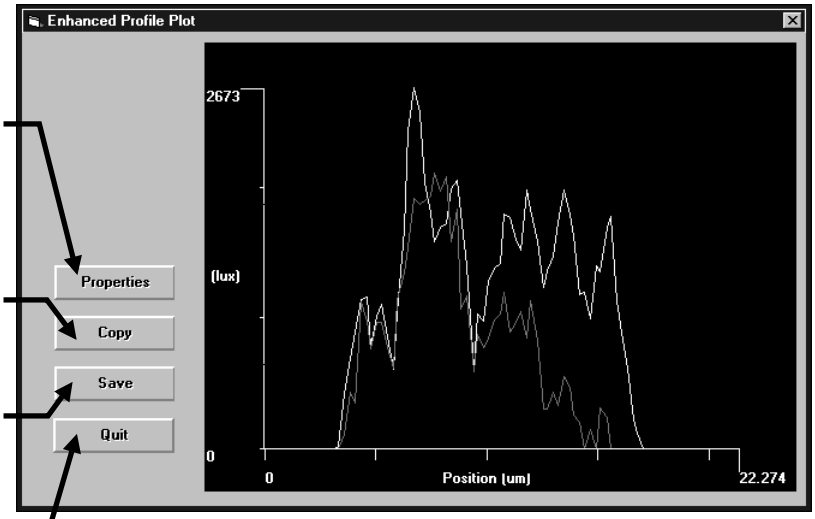


Fig. 2-108 [Enhanced Profile Plot] Window

TIP When a desired area is specified by dragging mouse left button over the graph, from upper left to lower right, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.
See section 2-11, "Transferring Data to Another Application" for details.

2-7-1-2 Intensity Values on a Planar Region (Bird's Eye View)

The intensity values on a region in an image can be displayed graphically.

1. Display the [Single] sub-panel at the front.

2. Display the image window of the image to be subjected to the intensity checking at the front.

3. When the image was acquired in the multi-channel mode, select whether the multiple channels are analyzed simultaneously or only one channel is analyzed.
   To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be analyzed.
   Example) When only the Ch1 image is displayed, only the Ch1 image is analyzed.

<Display channel switch> button

TIP For the switching of channels, see section 2-5-3, "Switching the Display Channels".

<Annotate> button

4. Click the <Annotate> button in the toolbar. A list of buttons appear as shown below.

5. From the displayed buttons, click the <Rectangular> button, <Circle> button or <Polyregion> button.

6. Specify the region to be checked in the image in the image window.
They can be specified as described below.

<Rectangular> button

- To specify a rectangle:
  On the image, drag the mouse pointer along the diagonal line of the desired rectangle, from the top left corner to the bottom right corner.

<Circle> button

- To specify a circle or ellipse:
  On the image, assume a rectangle circumscribing the circle to be checked and drag the mouse pointer along the diagonal line between opposite corners of the rectangle.

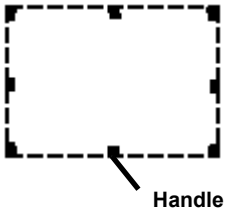
<Polyregion> button

- To specify a polygonal region:
  On the image, click the points corresponding to the corners of the polygon to be checked. After clicking the last corner point, click the right button of the mouse to connect the last clicked point to the first clicked point.

<Free Region> button

- To specify a free region:
  On the image, specify a region by dragging. Then release the mouse button to complete dragging. The point where the dragging was ended will be connected to the point where it was started.

A region is displayed on the image together with handles on the perimeter. The region is selected as the target of the bird's eye view while the handles are displayed.



NOTE

If the mouse is clicked in other place than inside the region specified on the image, the handles will disappear. The bird's eye view cannot be displayed while the handles are not displayed.

TIP

The checked region can be moved, deleted or changed of size or color. This is possible with the same method as entering comment in the image. For details, see sections 2-12-6, 2-12-7, 2-12-8 and 2-12-9 in section 2-12, “Entering Comment in Image”.

<Annotate> button

7. Click the <Annotate> button so that the list of buttons disappears.

8. Click the <Begin Analysis> button. The bird’s eye view of the specified region will be displayed in the [Intensity Profile] box of the [Analyze] panel.

TIP

The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the area (Area), horizontal and vertical lengths (X/Y, Z or T) and perimeter length (Perimeter) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

9. When the image was acquired in the multi-channel mode, the channel(s) to be subjected to the bird's eye view display can be selected using the [Channel] option buttons.

Shows:
Measurement results including;
· Perimeter
· Area
· X/Y, Z or T
Statistical channel data including;
· Total
· Average
· Std Dev

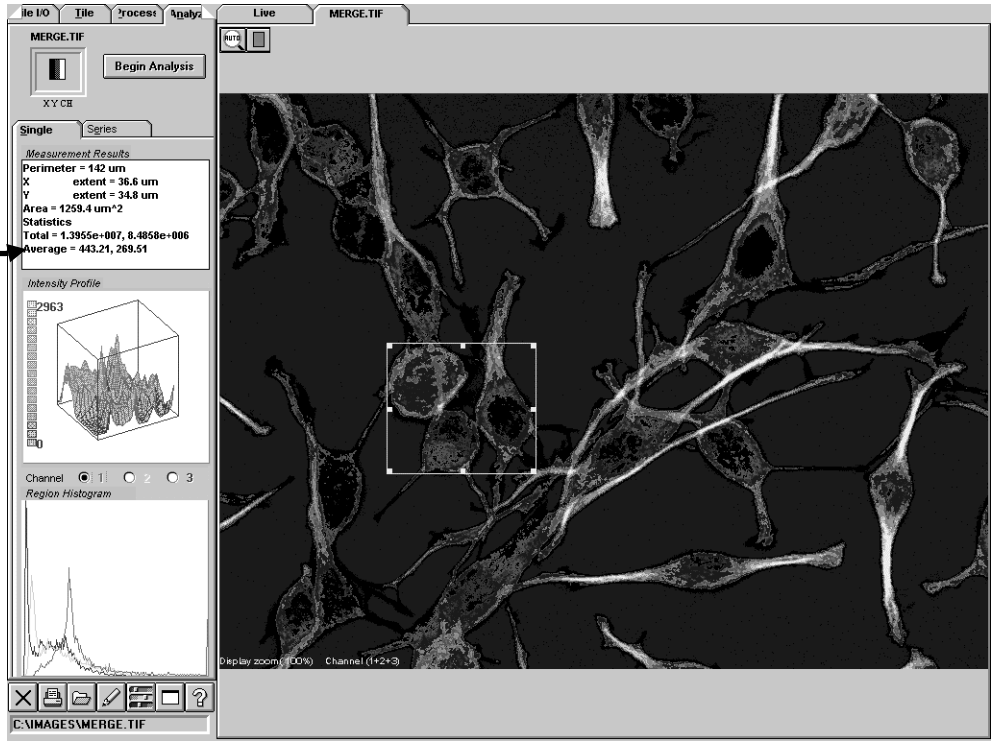


Fig. 2-109 Panel After Analysis (Region Specification)

10. Double-click the [Intensity Profile] button. The [Intensity Map] window appears as shown below.

[Angle] scale

Sets the angle in the horizontal direction.
The result can be confirmed with the small bird's eye view in the frame on the

[Tilt] scale

Sets the angle in the vertical direction. The result can be confirmed with the small bird's eye view in the frame on the top left.

<Plot> button

Displays the bird's eye view with the angles set above.

<Spin> button

Spins the bird's eye view by one turn. The spinning starts from the front.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze]

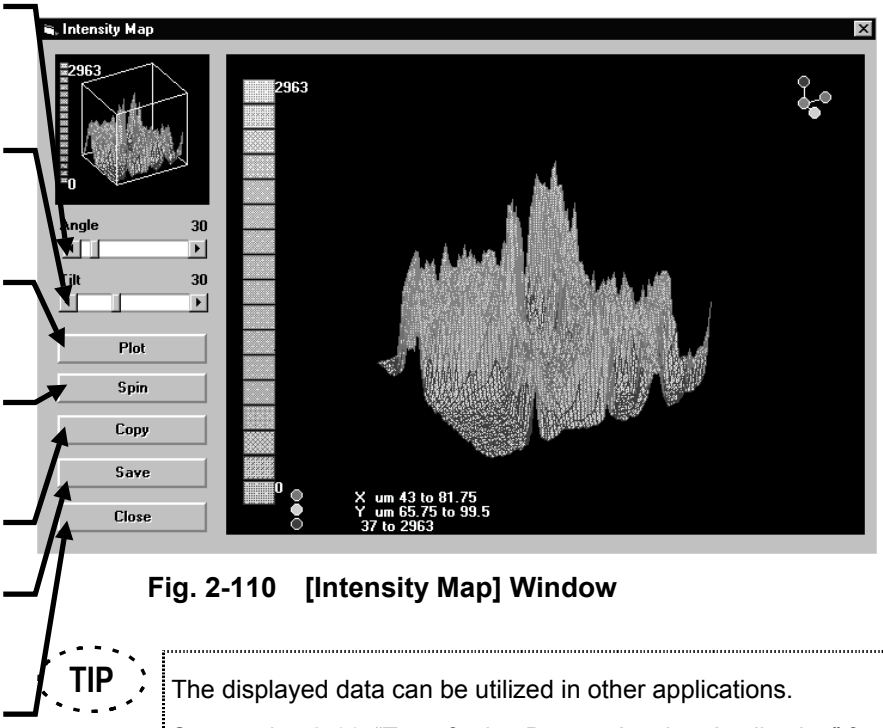


Fig. 2-110 [Intensity Map] Window

TIP

The displayed data can be utilized in other applications.

See section 2-11, "Transferring Data to Another Application" for details.

2-7-2 Checking the Intensity Distribution of a Specific Part

2-7-2-1 Intensity Distribution on a Line (Histogram)

The histogram on a line in an image can be displayed.

The histogram is displayed in the [Region Histogram] box in the [Single] sub-panel.

The operation method is identical to displaying the intensity profile on a line. See section 2-7-1-1, “Intensity Values on a Line (Line Profile)”.

Double-click the [Region Histogram] window. The [Enhanced Histogram Plot] windows appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

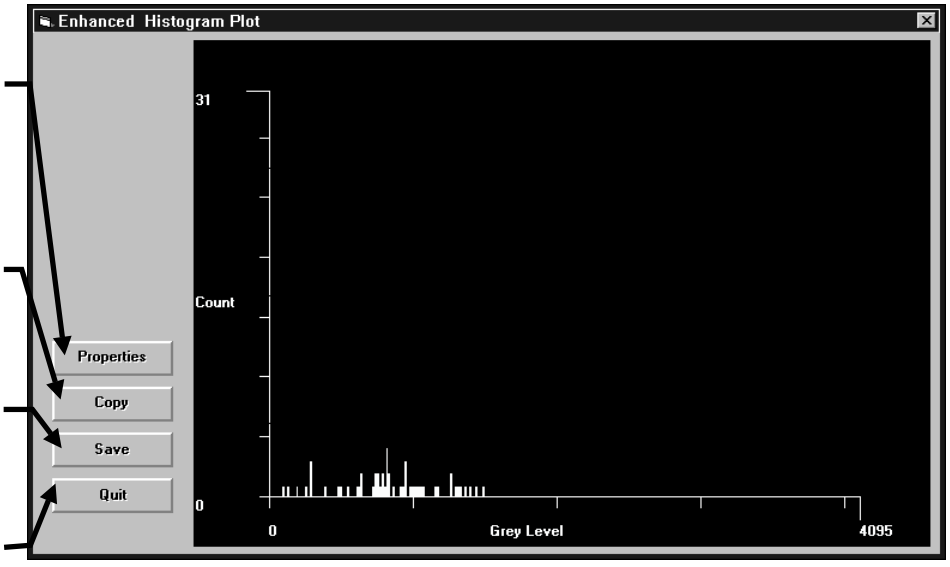


Fig. 2-111 [Enhanced Histogram Plot] Window (Line Specification)

TIP When a desired area is specified by dragging mouse left button over the graph, from upper left to lower right, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP

The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP

When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP

The displayed data can be applied to other applications.
See section 2-11, “Transferring Data to Another Application” for details.

2-7-2-2 Intensity Distribution on a Planar Region (Histogram)

The histogram on a region in an image can be displayed.

The histogram on a region in an image can be displayed.

The operation method is identical to displaying the intensity profile on a line. See section 2-7-1-1, “Intensity Values on a Line (Line Profile)”.

Double-click the [Region Histogram] window. The [Enhanced Histogram Plot] windows appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

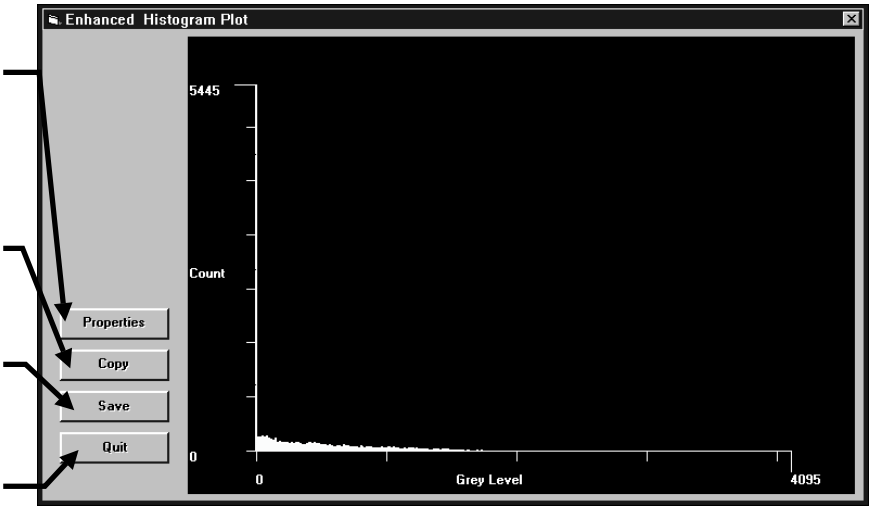


Fig. 2-112 [Enhanced Histogram Plot] Window (Line Specification)

TIP When a desired area is specified by dragging mouse left button over the graph, from upper left to lower right, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.
See section 2-11, “Transferring Data to Another Application” for details.

2-7-3 Image Measurement

2-7-3-1 Length Measurement

The length between two points in an image or the perimeter of a region in an image can be measured.

The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the length between 2 points (Length) or perimeter of the region (Perimeter) and the horizontal and vertical lengths (X/Y, Z or T) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

The operation method for measuring the length between 2 points is identical to that for displaying the intensity profile of a line. See section 2-7-1-1, “Intensity Values on a Line (Line Profile)”. The operation method for measuring the perimeter of a region is identical to that for displaying the bird’s eye view of a region. See section 2-7-1-2, “Intensity Values on a Planar Region (Bird’s Eye View)”.

TIP

The measurement results can be written in comment by copying and pasting them in the [Image Comments] dialog box in the [Experiments in Memory] dialog box.
See section 2-3-4, “Saving Comment Together with Image” for details.

2-7-3-2 Area Measurement

The area of a region in the image can be measured.

The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the area of the region (Area), perimeter of the region (Perimeter) and the horizontal and vertical lengths (X/Y, Z or T) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

The operation method is identical to that for displaying the bird’s eye view of a region. See section 2-7-1-2, “Intensity Values on a Planar Region (Bird’s Eye View)”.

2-7-3-3 Measuring the Change in Mean Value of Intensity

The mean value of the intensity in a region specified in an image can be measured and displayed graphically.

<Analysis-Series> button

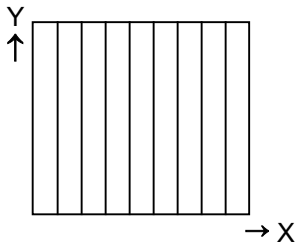
1. Select <Analysis-Series> button on tool bar and bring [Series] sub-panel.
2. Display the image window of the image that you want to check the intensity at the front.
3. When the image is composed of multiple image slices, operations will be applied to each slice of the image. When the image is composed of a single image slice or when one of the multiple image slices is selected, the [Single Slice Integration] group box appears as shown below. Select the direction of interest (X or Y) using the option buttons.

NOTE

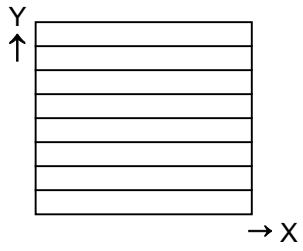
When the selected image was acquired in the XY observation mode, the [Single Slice Integration] group box shows the option buttons for selection of the X- or Y-direction. When the selected image was acquired in the XT observation mode, the option buttons for selection of the X- or T-direction are displayed.

Example) When the direction of interest is selected in the [Single Slice Integration] group box, line-by-line computation operation starts on the perpendicular lines to the selected direction. For example, an image acquired in XY observation is checked as shown below.

(When the X-direction is selected)



(When the X-direction is selected)



<Set start position> button

<Set end position> button

<Display>button

<XYZ series> button

<XYT series> button

<Display channel switch> buttons

TIP

When the image is composed of multiple image slices, the range of image slices to be subjected to the operation can be set using the <Set start position> and <set end position> buttons above the images. First display the image slice to start the operation using the <Display> button and click the <Set start position> button. Then, set the image slice to end the operation in the same way as above.

TIP

With an image acquired in XYZT observation, the slice images (cross-section (Z)/time lapse (T)) to be subjected to the operation can be selected using the <XYZ series> and <XYT series> buttons above the image.

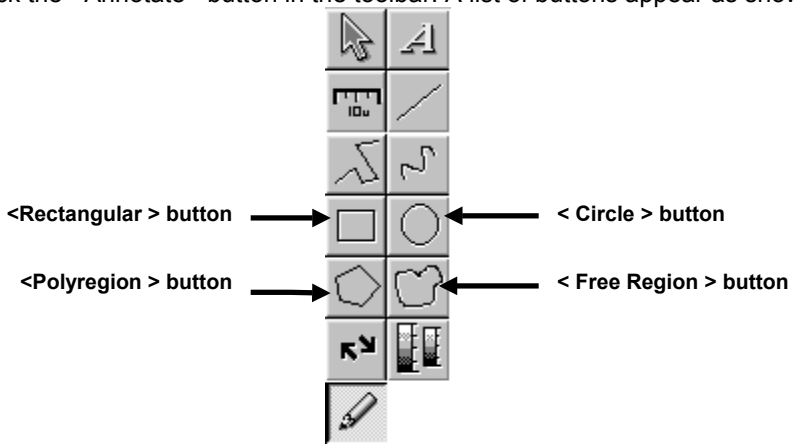
4. When the image was acquired in the multi-channel mode, select whether the multiple channels are operated simultaneously or only one channel is operated.
   To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be analyzed.
   Example) When only the Ch1 image is displayed, only the Ch1 image is analyzed.

TIP

For the switching of channels, see section 2-5-3, “Switching the Display Channels”.

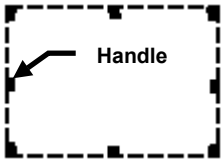
<Annotate> button

5. Click the <Annotate> button in the toolbar. A list of buttons appear as shown below.



6. From the displayed buttons, click the <Rectangular> button, <Circle> button, <Polyregion> button or <Free Region> button.

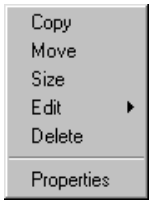
7. Specify the region to be checked in the image in the image window.
   For the specification method, see section 2-7-1-2, “Intensity Values on a Planar Region (Bird’s Eye View)”.
   A region is displayed on the image together with handles on the perimeter. The region is selected as the target of mean value computation operation while the handles are displayed.



NOTE

If the mouse is clicked in other place than inside the region specified on the image, the handles will disappear. On the contrary, clicking the mouse in a region displays handles around it. The operation cannot be executed while the handles are not displayed.

8. Click the mouse. A pop-up menu as shown below appears. Select [Properties] from the menu.



9. The [Properties] dialog box as shown below appears. Display the [Color] panel at the front.

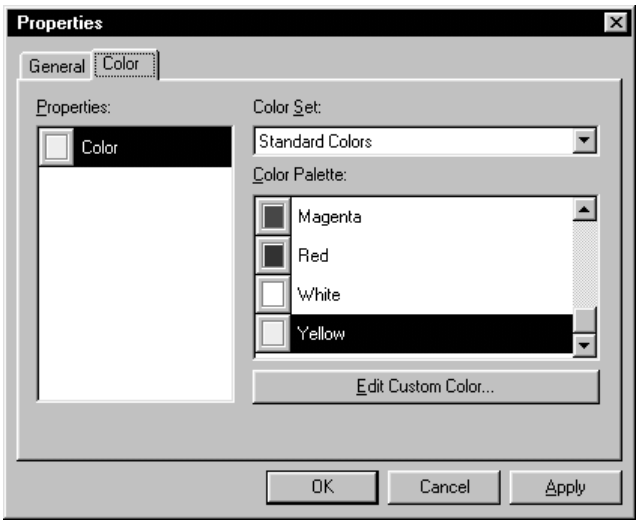


Fig 2-113 [Properties] Dialog Box

10. Select the desired color from the [Color Palette] list box.
11. It is also possible to specify more than one region simultaneously and display their operation results together. First specify the regions by repeating steps 7 and 8 above for each. Use different colors for the regions. After having set the regions, click the mouse in the first region. With the second regions and after, click the mouse while pressing the Shift key depressed.

<Annotate> button

12. Click the <Annotate> button so that the list of buttons disappears.
13. Using the scale in the [Threshold] group, set the threshold value for the intensity values used in operation. The intensity data above the threshold values set here will be used in the operation.

14. Click the <Begin Analysis> button. The mean value of the specified regions will be displayed graphically in the [Mean Intensity] box.

NOTE

The colors of the chart lines corresponding to the colors assigned to the regions.

NOTE

When the image was acquired in the multi-channel mode, the channel number is displayed to the right of each chart line.

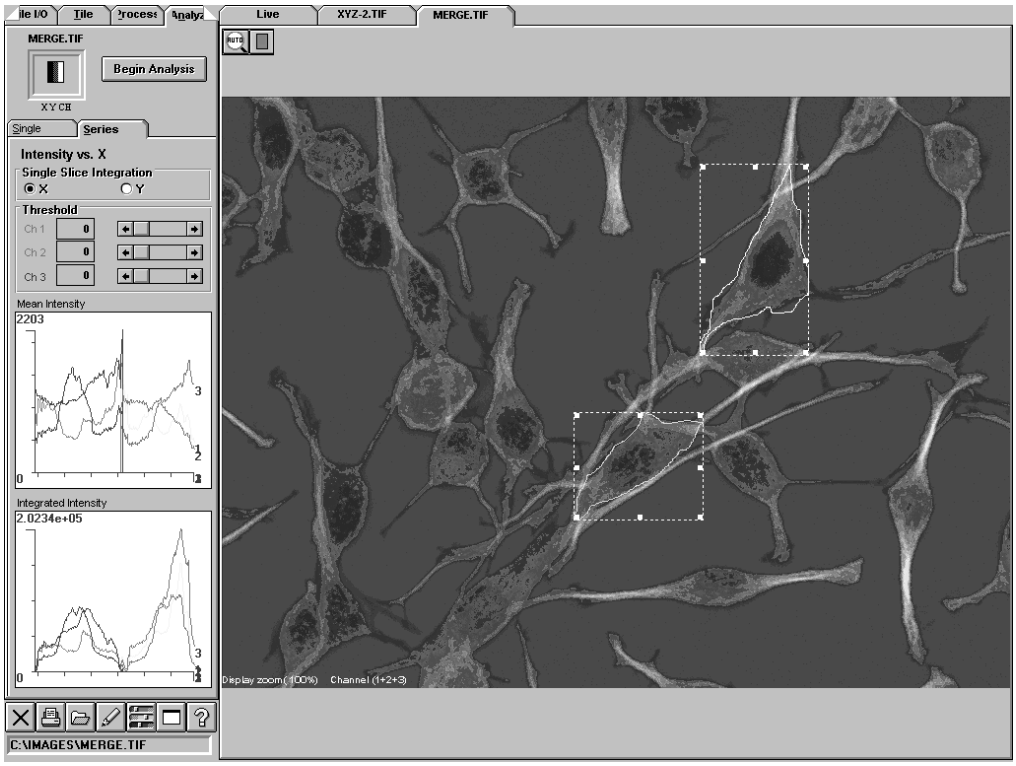


Fig. 2-114 Panel After Analysis

15. Double-click the [Mean Intensity] box. The [Average Intensity Trace] window appears as shown below

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Average Intensity Trace] window and returns to the [Analyze] panel.

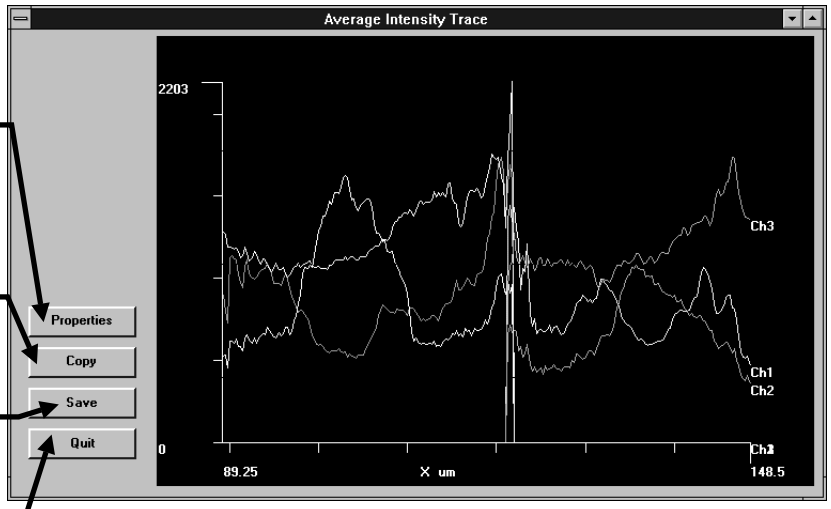


Fig. 2-115 [Average Intensity Trace] Window

TIP When a desired area is specified by dragging mouse left button over the graph, from upper left to lower right, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.
See section 2-11, “Transferring Data to Another Application” for details.

2-7-3-4 Measuring the Change in Integrated Intensity

The total value of the intensity in a region specified in an image can be measured and displayed graphically.

The operation results are displayed graphically in the [Integrated Intensity] box in the [Series] sub-panel.

The operation method is completely identical to that for obtaining the mean value of intensity. See section 2-7-3-3, “Measuring the Change in Mean Value of Intensity”.

Double-click the [Integrated Intensity] box. The [Integrity Intensity Trace] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Integrated Intensity Trace] window and returns to the [Analyze] panel.

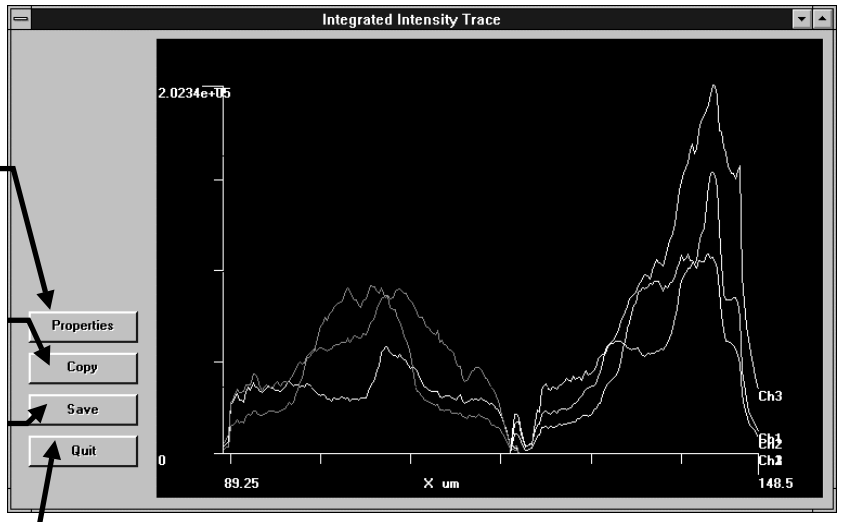


Fig. 2-116 [Integrated Intensity Trace] Window

TIP When a desired area is specified by dragging mouse left button over the graph, from upper left to lower right, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP

The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP

When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP

The displayed data can be applied to other applications.
See section 2-11, “Transferring Data to Another Application” for details.

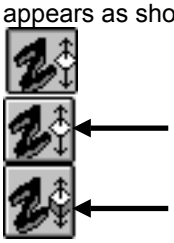
2-8 Building an Image from a Different Viewpoint

2-8-1 Building Extended Focus Image from XYZ Image

2-8-1-1 Display Switching to Built Image

An extended focus image can be built from XYZ (multiple sections) images and the display can be switched to show the built image.

1. Display the image window of the XYZ (multiple sections) image.
2. The following button is displayed at the top of the image window.
   (Usually, only the <XYZ series> button is displayed. When it is clicked, a list of buttons appears as shown below.)



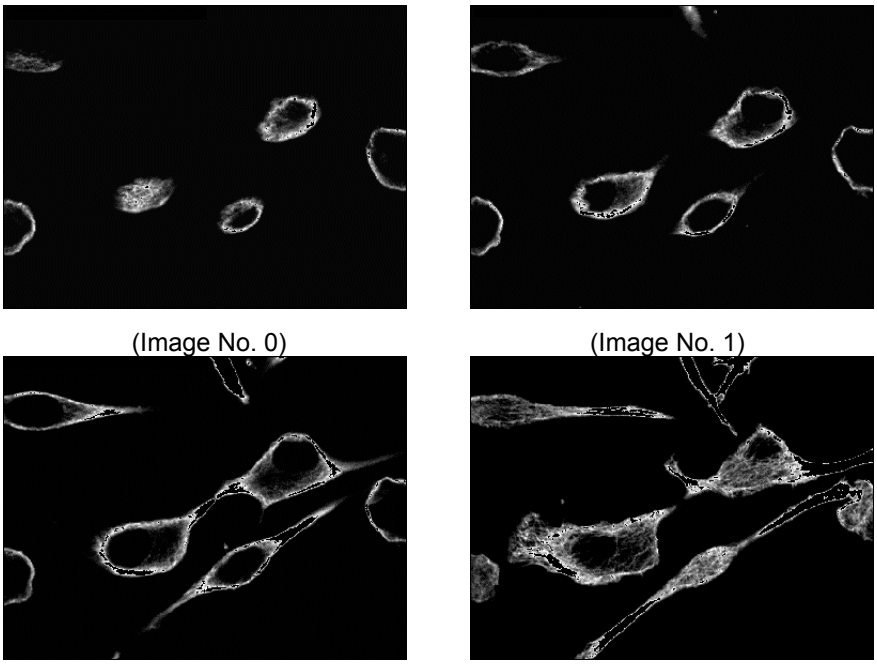
<XYZ series> button
Displays only one of multiple sections image slices.

<Extend> button
Displays the extend image.

3. Click the <Extend> button.

<Display>button

4. Click the <Display> button at the top of the image window repeatedly to build the extended-focus image.
   (The extended-focus image can also be displayed by clicking and holding the <display> button for successive display.)



(Image No. 0) (Image No. 1)

(Image No.2) (Image No. 3)

Fig. 2-117 Four Images Used in Building Extended-Focus Image

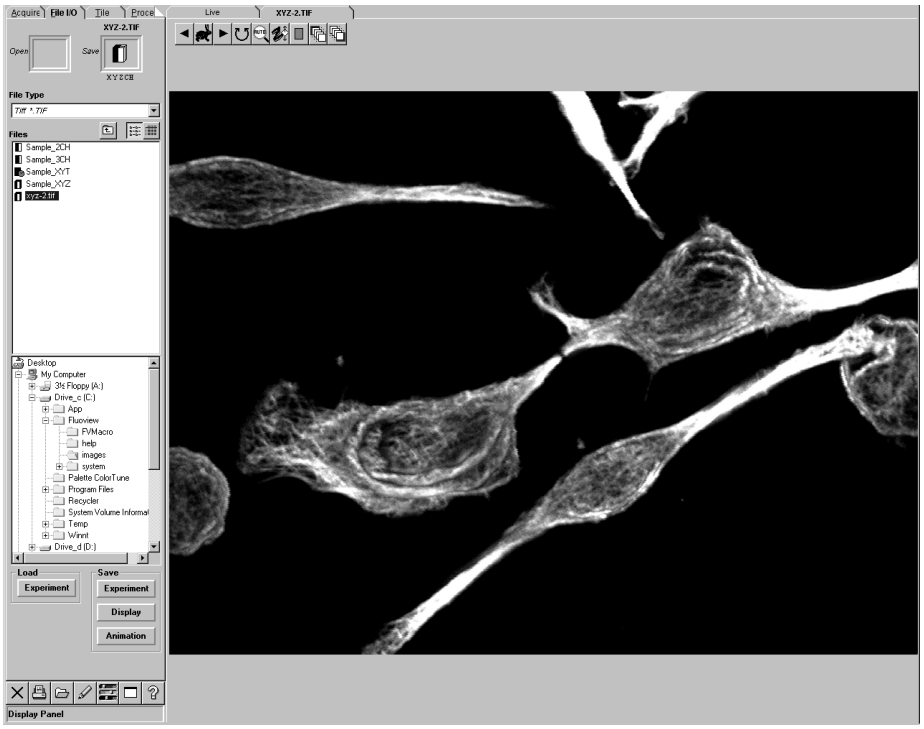


Fig. 2-118 Panel Showing an Extended-Focus Image

Among the multiple image slices composing the XYZ image, the range of image slices to be used in extend image building can be specified.

1. Display the image slice to be set as the start image by using the <Frame advance> or <Successive display> buttons at the top of the image window.

<Set start position> button

2. Click the <Set start position> button at the top of the image window.

3. Display the image slice to be set as the end image by using the <Frame advance> or <Successive display> buttons at the top of the image window.

<Set end position> button

4. Click the <Set end position> button at the top of the image window.

NOTE **The <Set start position> and <Set end position> buttons are valid in the pushed-in condition. To cancel a previously set start or end position, click the <Set start position> or <Set end position> button again.**

NOTE **When it is required to analyze the built extended-focus image or save it in the Fluoview Multi Tiff format, create the extended-focus image as a separate image from the original image. See section 2-8-1-2, "Turning Built Image into Single Image".**

2-8-1-2 Turning Built Image into Single Image

From XYZ (multiple sections) image, an extended-focus image can be built as a separate image from the original image.

Use the [Visualize] panel to build the image.

First display the [Visualize] panel.

image window
Shows the image. The file name of the image is shown in the page tab of the panel.

[Begin Visualize] button
Starts building the extended-focus image.

[Rendered View] group box

[Extended Focus View] option button
Builds the extended-focus image.

[Arbitrary View] option button
Builds the image for stereo display.

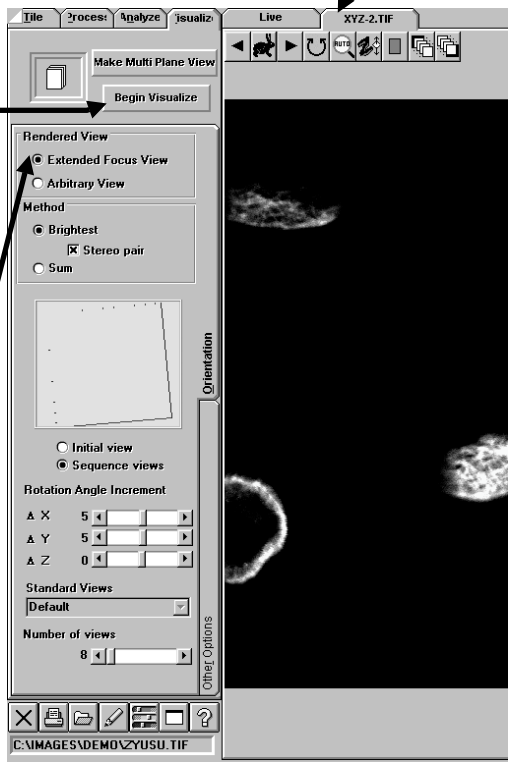


Fig. 2-119 [Visualize] Panel

1. Display the image window of the XYZ (multiple sections) image.

2. Click the [Extended Focus View] option button in the [Rendered View] group box.

3. Click the <Begin Visualize> button to start the image building. When it completes, the built image is displayed in the [Extended] panel.

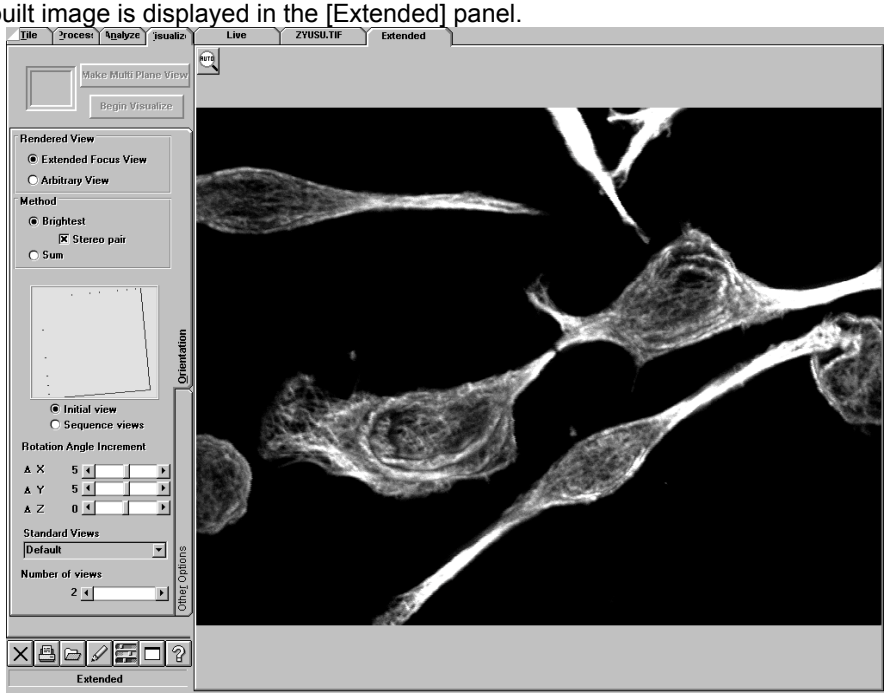


Fig. 2-120 Panel Showing Extended-Focus Image

2-8-1-3 Turning Built Image into time series image

From XYZT image, an extended-focus image can be built as a separate time series image from the original image.

Use the [Visualize] panel to build the image.

First display the [Visualize] panel.

1. Display the image window of the XYZT image.

2. Click the [Extended Focus View] option button in the [Rendered View] group box.

3. Click the <Begin Visualize> button to start the image building. When it completes, the built image is displayed in the [Extended] panel.

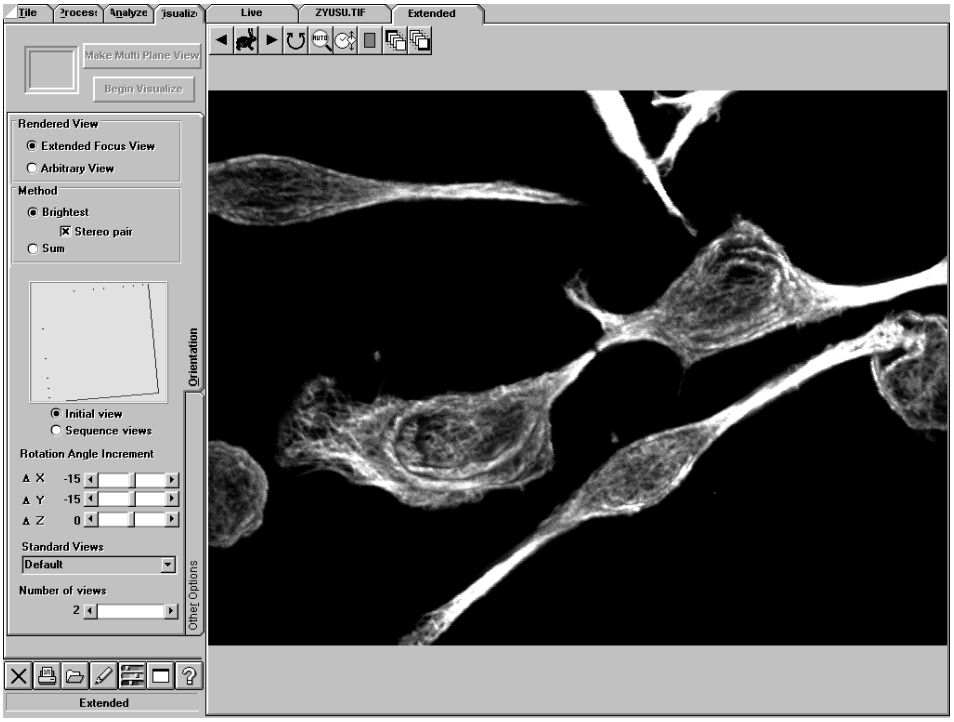


Fig. 2-121 Panel Showing Extended-Focus Image

2-8-2 Building line images to be viewed in Z direction

The images acquired by cutting the XYZ image off vertically and horizontally can be displayed with the information on each line.

Each line images is created as a separate image from the original one.

1. Display the image window of the XYZ (multiple sections) image.
2. Click the < New Multi-Plane Form > button in the toolbar.

   The [3D-] panel is created to start the line image building.

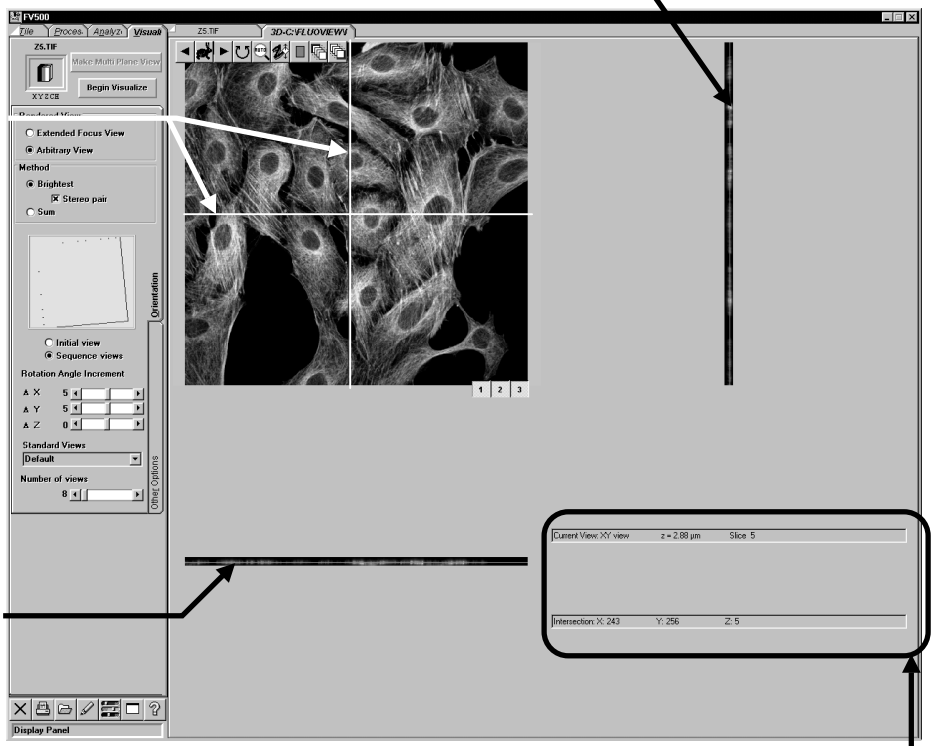
< New Multi-Plane Form > button

Line image display in YZ-direction

Display the image of the line specified on the XY-direction image.

Image display in XY-direction

The line image to be displayed in XZ or YZ-direction can be moved by dragging the red line (XZ) or brown line (YZ) on the XY-direction image.



Line image display in XZ-direction

Display the image of the line specified on the XY-direction image.

Fig. 2-122 Panel showing the line images to be viewed in Z direction

Upper row : shows the current slice and the steps in Z-direction acquiring the image.

[z=] : the number of current steps

[Slice] :the number of current slice

Lower row: shows the current X and Y coordinates and Z position on the XY-direction image.

[X:] : current X position

[Y:] : current Y position

[Z:] : current Z position

<Display> button

TIP

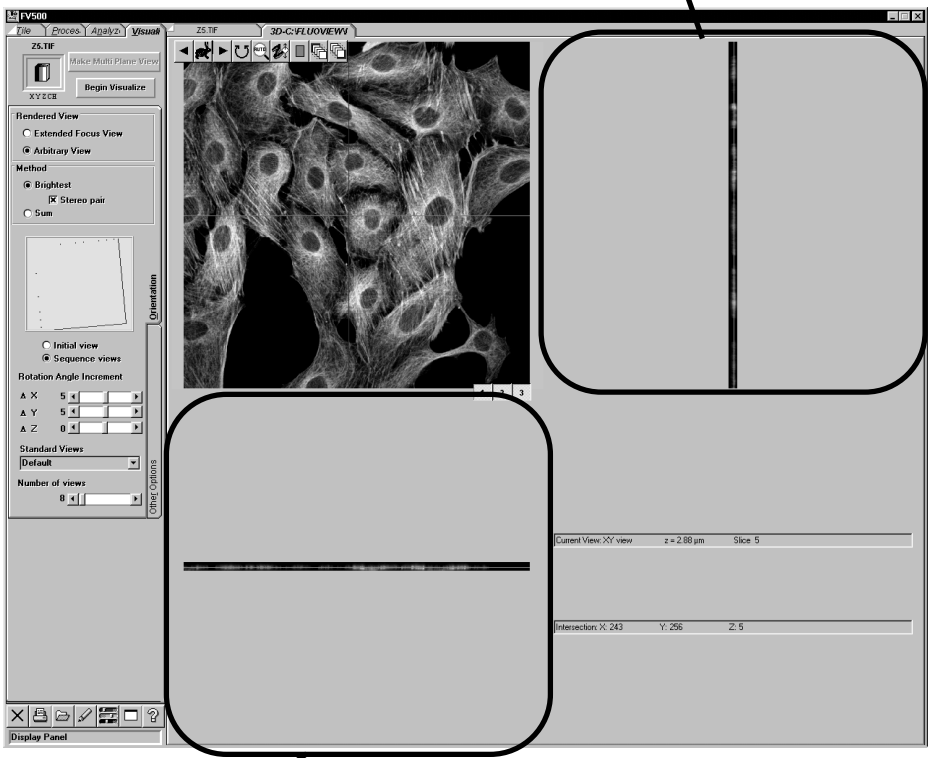
If you change the slice to be displayed with the <Display> button, [Slice] and [Z:] aren't effected.

[Slice] and [Z:] show current slice and Z position according to moving the red or brown line indicating XZ or YZ-direction on the XY-direction image displayed left above.

Image in XZ or YZ on 3D image built can be cut out and displayed.

1. Click any part inside the image that appears in XZ or YZ direction on [3D] panel.

Click inside part of this place in case that the image in YZ is cut out and displayed.



Click inside part of this place in case that the image in XZ is cutout and displayed.

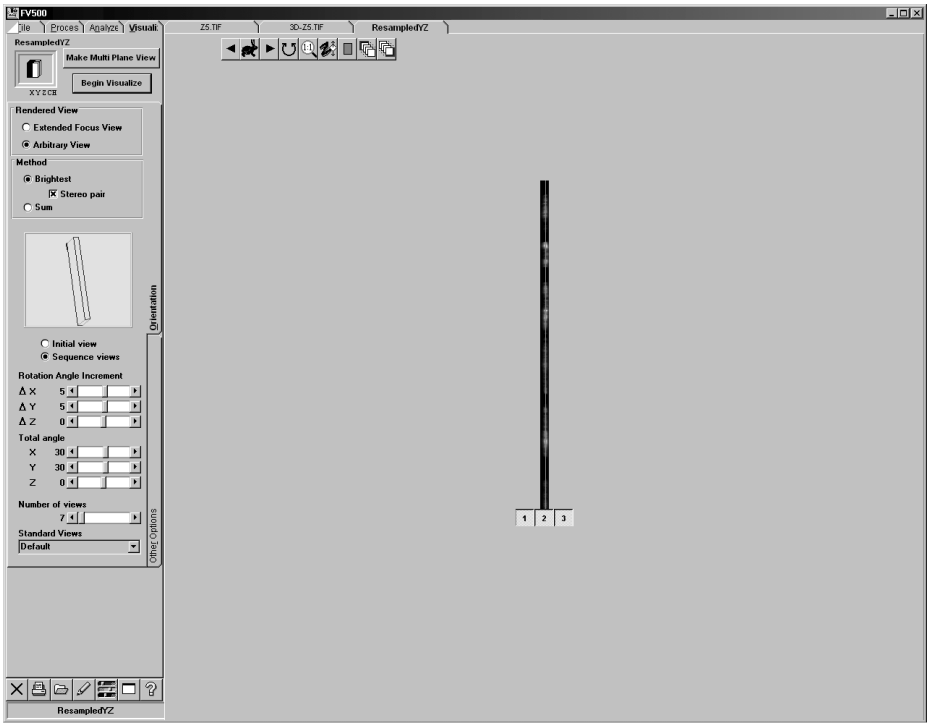
TIP

Even if inside part of the image that appears in XY direction on [3D] panel is clicked, <Accept this view as new experiment> will not be turned valid. The part inside the image in XZ or YZ direction must be clicked.

<Accept this view as new experiment> button

2. Select <Accept this view as new experiment> button on [Visualize] panel.

TIP In case of Ver5.0, select <Make experiment from the X-Z view> button or <Make experiment from the Y-Z view> button.



One Point!

This above illustration indicates Ver4.3a image.

In case of Ver5.0, 3D image based on XZ or YZ direction can be built.

One Point!

When building 3D image in Ver5.0 or subsequent version, pay attention to the following points.

When using XZ direction as reference, 3D image including images of YZ (upper right) and XY (lower left) can be built.

When using YZ direction as reference, 3D image including images of XY (upper right) and XZ (lower left) can be built.

In this case, the size of image in each direction may be different.

The XY display area in the 3D image is intended for life-size display of image pixel values. The X and Y resolution values are not taken in consideration at this time.

However, when both of the following conditions are met, Z in the XZ and YZ display areas are displayed in a larger size than the actual pixel value.

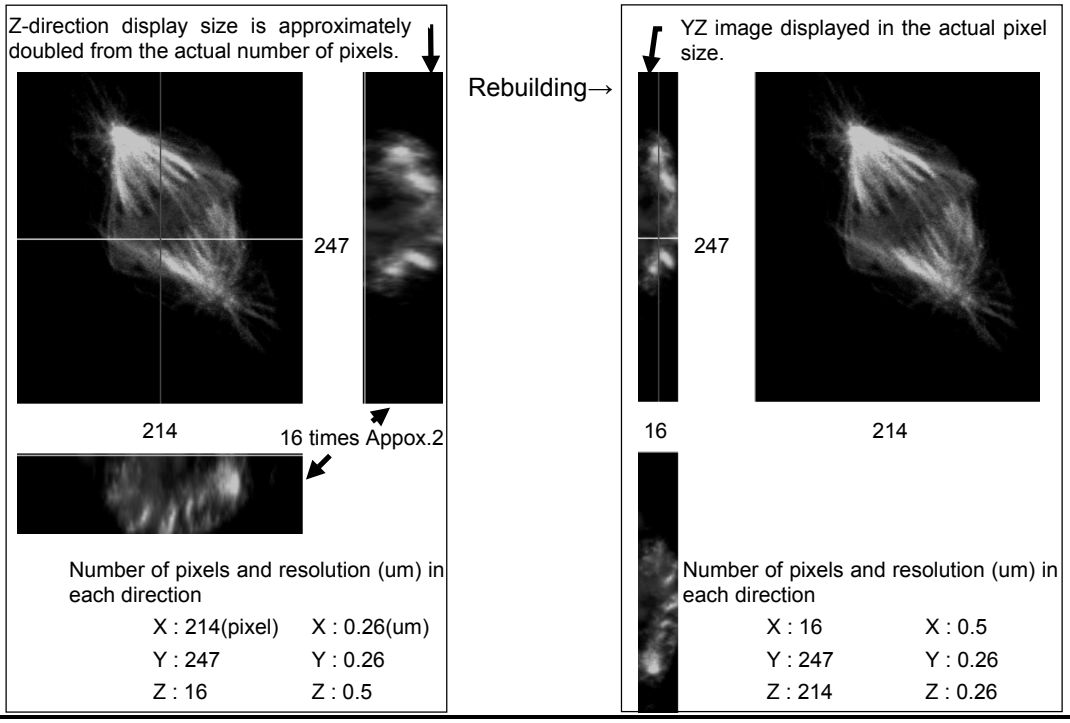
· Resolutions of X and Y are equal

· The resolution of Z is higher than that of X and Y.

Example: Rebuilding the 3D image of Sample_XYZ.tif based on its YZ image.

Left figure (Before building): Resolutions of X and Y are equal (both 0.26) and the resolution of X (0.5) is higher than that of Y, Z is displayed in a larger size than the actual number of pixels. (Since the resolution of Z is about twice that of X and Y, the display in the Z direction is approximately doubled compared to the actual size.)

Right figure (After building): Resolutions of X(0.26) and Z(0.5) are changed with each other. As a result, resolutions X and Y are unequal so the image is displayed in the actual pixel size.


Z-direction display size is approximately doubled from the actual number of pixels.
247
214
16 times Appox.2
Number of pixels and resolution (um) in each direction
X : 214(pixel) X : 0.26(um)
Y : 247 Y : 0.26
Z : 16 Z : 0.5
Rebuilding→
YZ image displayed in the actual pixel size.
247
16
214
Number of pixels and resolution (um) in each direction
X : 16 X : 0.5
Y : 247 Y : 0.26
Z : 214 Z : 0.26

2-9 Viewing 3D Image

Use the [Visualize] panel to view an image three-dimensionally.

First display the [Visualize] panel.

[Begin Visualize] button

Starts building the images for 3D display.

[Method] group box

(Explanation when the [Arbitrary View] option button is selected)

[Brightest] option button

Builds the image by accumulating the intensity value.

[Stereo Pair] check box

To be checked when building a pair of stereo 3D images or a 3D image to be viewed through color (red/green) eyeglasses.

[Sum] option button

Builds the image by adding the

[Initial view] option button

Sets the angle at which the rotation should start. The angle itself can be set using the [Initial Rotation Angle] scale immediately below the option button.

[Sequence views] option button

It is selected to determine per what degree the view should be rotated. The [Rotation Angle Increment] scale and the [Total angle] scale appear as shown in lower right figure and each angle factor can be set.

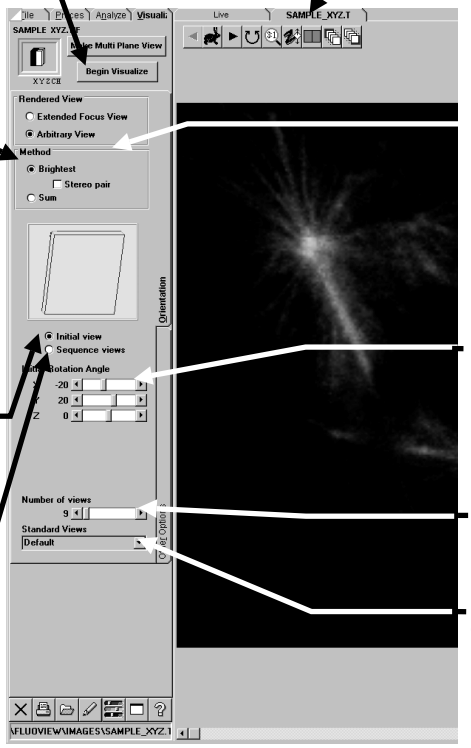


image window

Shows the image. The file name of the image is shown in the page tab of the panel.

[Rendered View] group box

[Extended Focus View] option button

Checking this option button changes the check box in the [Method] group as shown in the lower left figure.

[Arbitrary View] option button

Builds the image for 3D display.

[Initial Rotation Angle] Scale

This appears when [Initial View] option button is selected. Angle can be set.

[Rotation Angle Increment] scale

Set the angles

[Standard Views] drop-down list

Selects the rotation direction. It is possible to set an arbitrary direction.

[Number of views] scale

Sets the number of views displayed during rotation.

Fig. 2-125 [Visualize] Panel and [orientation] Sub-panel

[Method] Group Box

When [Extended Focus View] option button is selected, [Method] group box will change as shown in figure.

[Intensity Projection] Option Button

Builds an image that focused on each cross section as shown in Fig. 2-126.

[Topographic Projection] Option Button

Builds an image that indicates which cross section the focal point is located as shown in Fig. 2-127.

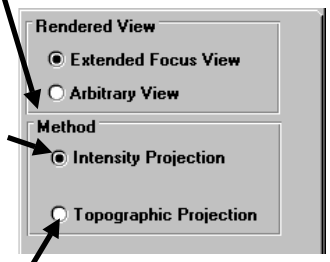


Fig 2-123 when [Extended Focus View] option button is selected

[Rotation Angle Increment] Scale

Set an angle increment for the view rotation.

[Total angle] Scale

Set the total angle from 1st view to the last one.

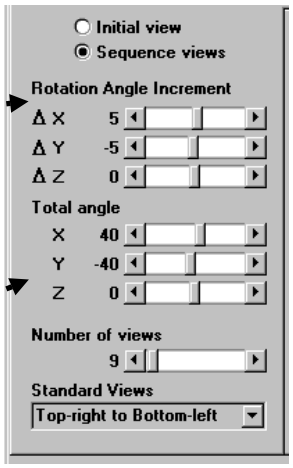


Fig 2-124 when [Sequence Views] option button is selected

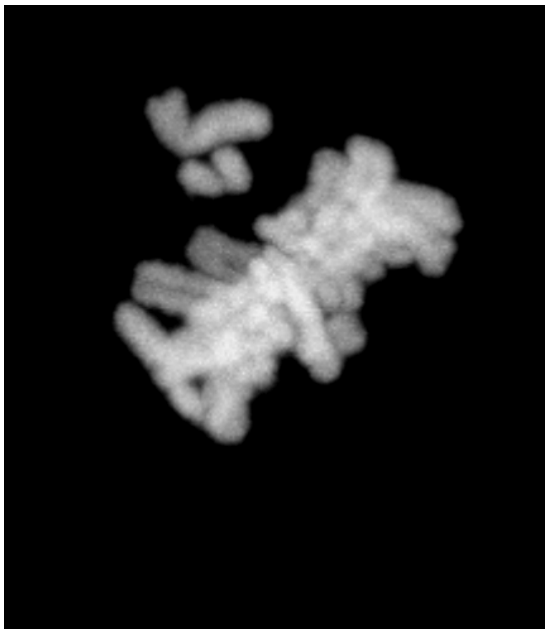


Fig 2-126 Example of Intensity Projection

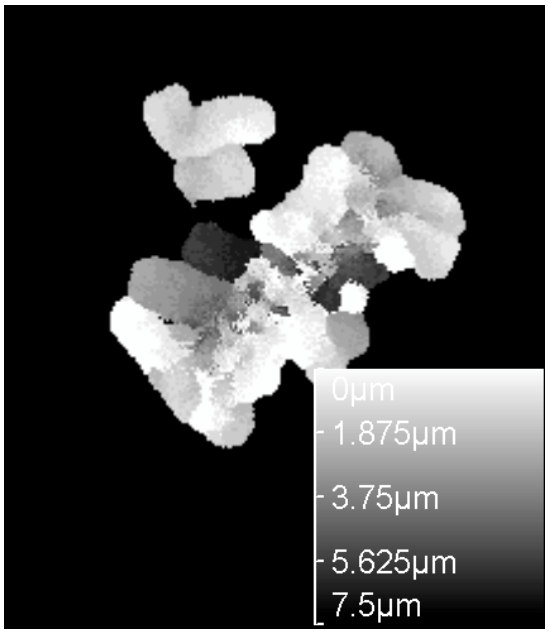


Fig 2-127 Example of Topographic Projection

[Threshold range Low / High] scale

Sets the range of intensity values to be used in image building.

[Depth Weight] scale

Sets the weighting in the depth direction. By increasing this setting, it is possible to provide the image with a perspective by darkening the far objects and brightening near objects.

[Stereo Factor] text box

Sets the deviation between the left and right eyes when building a pair of stereo 3D images or a 3D image to be viewed through color (red/green) eyeglasses.

[Z stretch Factor] text box

Provides each multiple sections image with a feeling of thickness. The value displayed here in advance has been calculated by the system so that the scale in the planar, or XY-direction of the image is identical to the scale in the depth, or Z-direction. Usually, this value does not need to be changed.

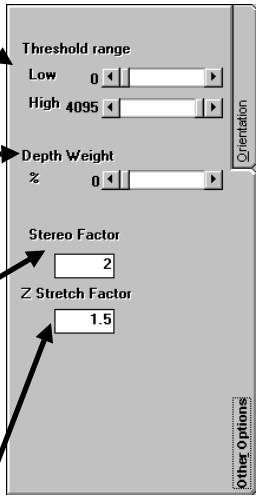
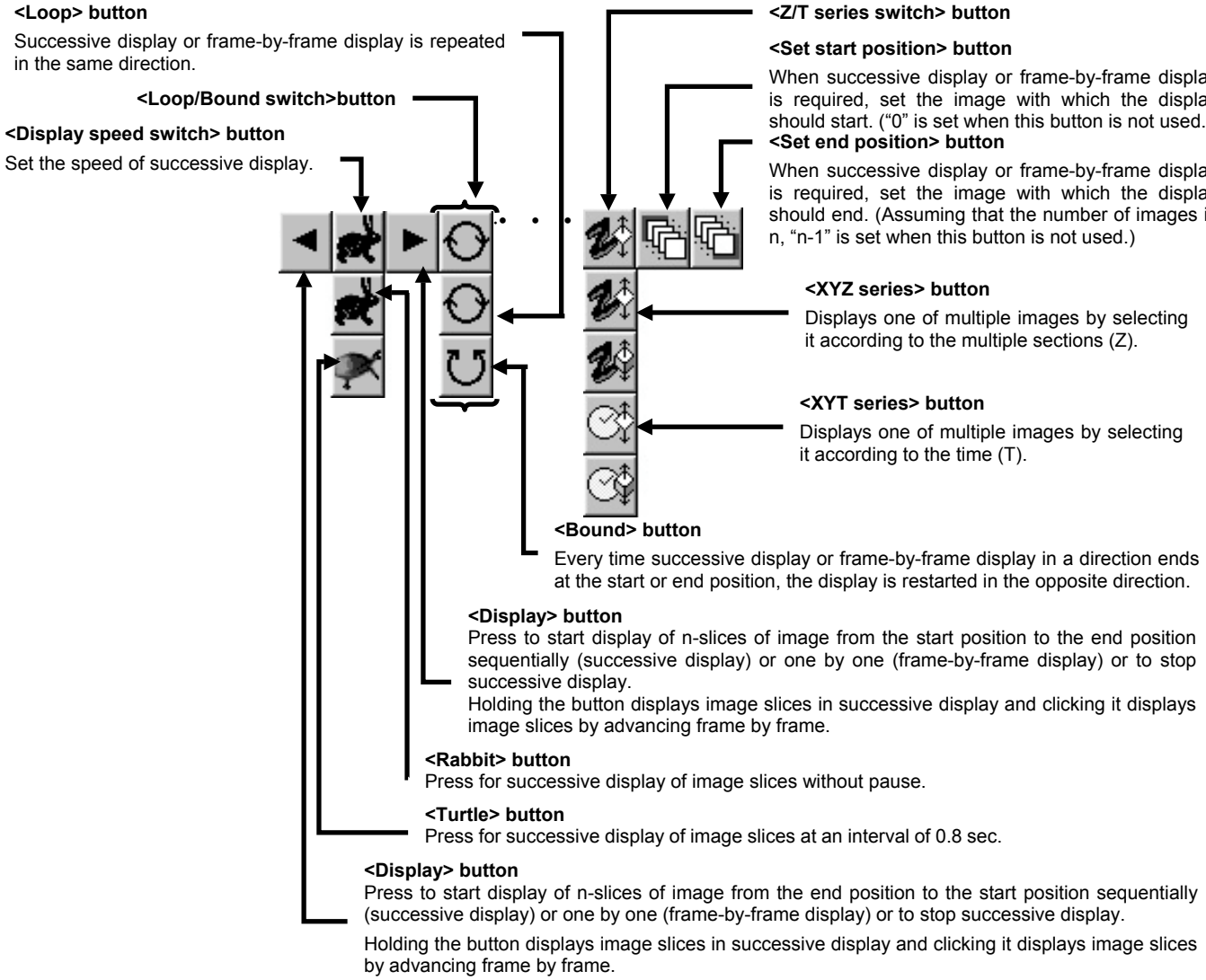


Fig. 2-128 [Other options] Sub-panel of [Visualize] Panel

2-9-1 Successive Display of Images

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation, XYZT observation) can be displayed successively using the buttons at the top of the image window as shown below.

1. Display the image window of the image composed of multiple image slices.
2. The buttons as shown below are displayed at the top of the image window. If the <XYZ series> button is not displayed under the <Z/T series switch> button while the images were acquired in XYZT observation mode, click the <Z/T series switch> button and click the <XYZ series> button in the displayed list of buttons.

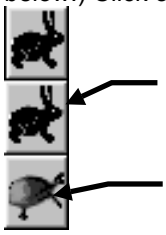

<Loop> button
Successive display or frame-by-frame display is repeated in the same direction.
<Loop/Bound switch>button
<Display speed switch> button
Set the speed of successive display.
<Z/T series switch> button
<Set start position> button
When successive display or frame-by-frame display is required, set the image with which the display should start. ("0" is set when this button is not used.)
<Set end position> button
When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, "n-1" is set when this button is not used.)
<XYZ series> button
Displays one of multiple images by selecting it according to the multiple sections (Z).
<XYT series> button
Displays one of multiple images by selecting it according to the time (T).
<Bound> button
Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.
<Display> button
Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.
<Rabbit> button
Press for successive display of image slices without pause.
<Turtle> button
Press for successive display of image slices at an interval of 0.8 sec.
<Display> button
Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

3. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
4. Display the image slice to end the successive display by using the <Frame advance> button at the top of the image window.
5. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming that the number of images is n, becomes the start image automatically.)
6. Click the <Successive display> button. The images will be displayed successively from the start position to the end position.
   Click the <Stop> button to stop the successive display.

2-9-1-1 Changing the Successive Display Speed

The buttons on the top of the image window can be used to vary the speed of successive display of multiple image slices.

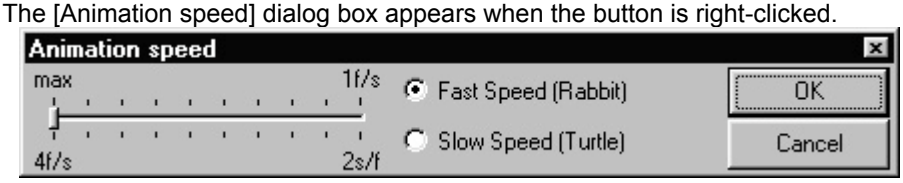
1. Display the image window of the image to be subjected to successive display speed change.
2. The buttons as shown below are displayed at the top of the image window.
   (Usually, the <Rabbit> button is displayed. Clicking it displays a list of buttons as shown below.) Click one of the buttons to select the display speed.



<Rabbit> button
For successive display without pause.

<Turtle> button
Press for successive display of image slices at an interval of 0.8 sec.

3. The display speed provided by the <Rabbit> or <Turtle> button can be varied by clicking the mouse right button on each button.
   The [Animation speed] dialog box appears when the button is right-clicked.



4. Select the option button of the speed to be varied.

5. Set the desired display speed in the scale on the right.

6. Click the <OK> button to close the [Animation speed] dialog box.

2-9-1-2 Changing the successive image display position

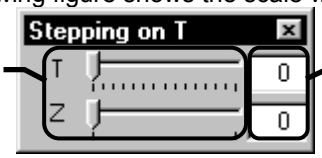
The successive display position of multiple image slices in an image can be changed using a bottom displayed at the top of the image window.

1. Display the image window of the image subjected to successive display position change at the front.

<Display> button

2. Click the mouse right button on the <Display> button at the top of the image window to display a scale. (The following figure shows the scale with an XYZT image.)

Drag the scale or click a point on it to change the display position to a position before or after the current position.



The display position can also be changed by direct entry of the value.

3. Drag the scale to another position to move the successive display position to the position.

   Entering the value directly in the text box can also move the display position.

2-9-2 Animation

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation, XYZT observation) can be built into animation image, which can be displayed in 3D by rotating images.

1. Display the image window of the image composed of multiple image slices.

2. When the images were acquired in the multi-channel mode, select whether animation is built from images of more than one channel or from an image of only one channel.
   To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be used in the animation building.
   Example) When only the Ch1 image is displayed, only the Ch1 image is used.

<Display channel switch> buttons

TIP

For the switching of channels, see section 2-5-3, “Switching the Display Channels”.

3. Click the [Arbitrary View] option button in the [Rendered View] group box.

4. Click the [Brightest] or [Sum] option button in the [Method] group box.

5. Select the image rotation direction from the [Standard Views] drop-down list.

6. Click the [Initial view] option button.

7. Set the angle at which the rotation should start using the [Initial Rotation Angle] scale.

8. Click the [Sequence views] option button.

9. Set the angle per rotation step using the [Rotation Angle Increment] scale.

10. Set the number of images to be rotating using the [Number of views] scale.

11. Click the <Begin Visualize> button to start building the animation. When the building completes, the built image is displayed in the [3D Animation] panel.

TIP The status bar shows the progress of building processing.

<Cancel Visualize> button

TIP During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

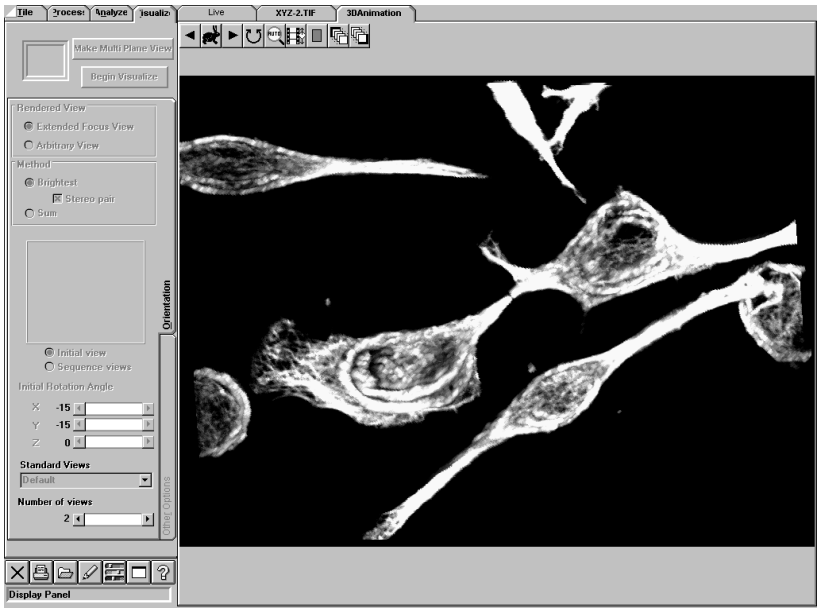


Fig. 2-129 Panel Showing the Built Image

<Display> button

12. Click and hold the <Display> button at the top of the [3D Animation] panel. The images will rotate so that they can be viewed three-dimensionally.

TIP Click the <Stop> button to stop the image rotation.

2-9-3 Building Stereo 3D Images

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation) can be built into a pair of stereo 3D images. These images can be viewed three-dimensionally by watching them with two eyes for a while.

The operation is similar with animation. Perform steps 1 to 10 in section 2-9-2, “Animation”, then proceed to the following steps. (Except that, in the [Method] group box, it is required to click the [Brightest] option button.)

1. Check the [Stereo Pair] check box.
2. Click the <Begin Visualize> button to start building the images. When the building completes, the built images are displayed in the [3D Animation] panel.

TIP

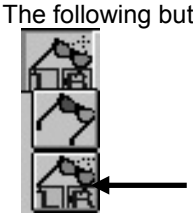
The status bar shows the progress of building processing.

<Cancel Visualize> button

TIP

During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

3. The following buttons are displayed at the top of the image window.



Stereo 3D images.

4. Watch the two images with two eyes for a while. They will look as a single 3D image.

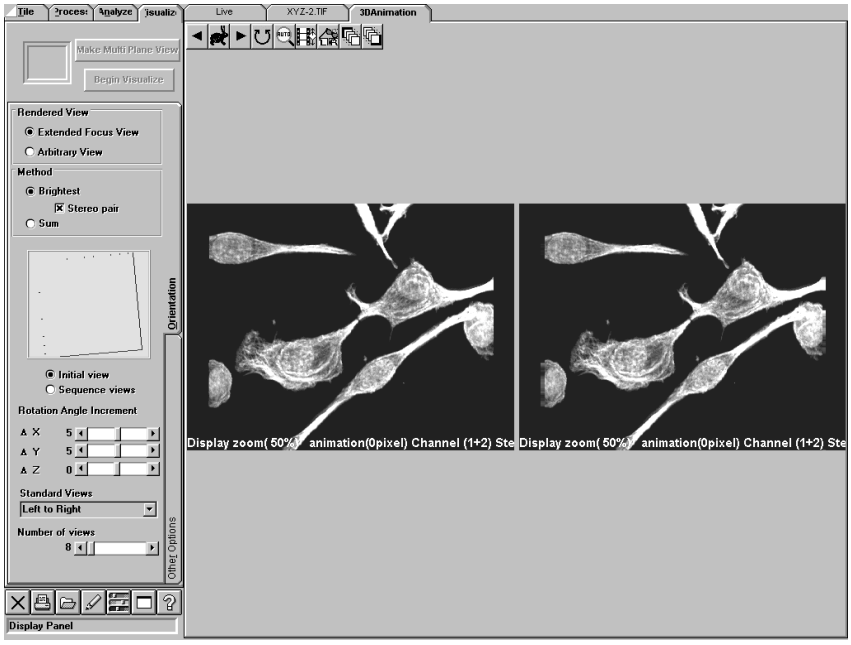


Fig. 2-130 Panel Showing Stereo 3D Images

2-9-4 Building a 3D Image to be Viewed Through Color (Red/Green) Eyeglasses

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation) can be built into an image which looks 3D when viewed through a pair of color (red/green) eyeglasses. This image can be viewed three-dimensionally by watching them with two eyes for a while.

The operation is similar with animation.. Perform steps 1 to 10 in section 2-9-2, “Animation”, then proceed to the following steps. (Except that, in the [Method] group box, it is required to click the [Brightest] option button.)

1. Check the [Stereo Pair] check box.
2. Click the <Begin Visualize> button to start building the images. When the building completes, the built image is displayed in the [3D Animation] panel.

TIP

The status bar shows the progress of building processing.

TIP

During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

3. The following buttons are displayed at the top of the image window.

3D image for color (red/green) eyeglasses.

4. Watch the image through a pair of color (red/green) eyeglasses.

TIP

The color (red/green) eyeglasses can also be used to view the animation. To start animation, click the <Successive display> button.

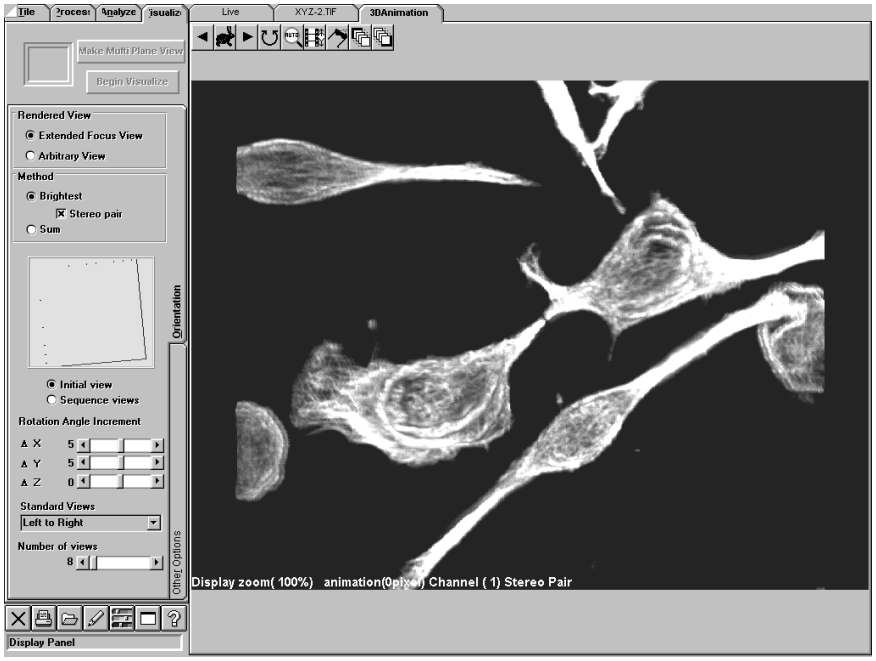


Fig. 2-131 Panel Showing 3D Image to be Viewed Through Color Eyeglasses

2-10 Viewing Images Following the Progress of Time

Images composed of multiple image slices (XYT, XYZT or XZT observation) can be displayed following the time lapse to show the change over time.

2-10-1 Displaying Images Together

The change over time can be viewed at a glance by displaying multiple image slices together. For the detailed operation method, see section 2-5-7, “Displaying Multiple Image Slices Together”.

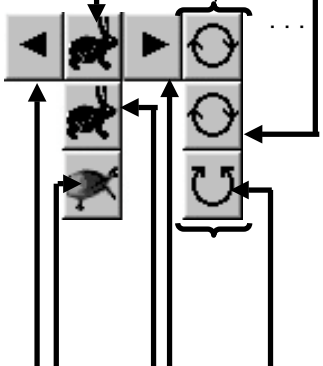
2-10-2 Displaying Images Successively

The change over time can also be displayed as animation.
With images composed of multiple slices, such as time-lapse images the buttons as shown below are displayed at the top of the image window.

1. Display the image window of the image composed of multiple image slices.

2. To display XYZT observation images by noticing the progress of time, it is required to select the <XYT series> button under the <Z/T series switch> button at the top of the image window. If the <XYT series> button is not displayed, click the <Z/T series switch> button and click the <XYT series> button in the displayed list of buttons.

3. Display the image slice to start the successive display by using the <Frame advance> button at the top of the image window.



<Loop> button

Successive display or frame-by-frame display is repeated in the same direction.

<Loop/Bound switch>button

<Display> button

Set the speed of successive display.

<Z/T series switch> button

<Set start position> button

When successive display or frame-by-frame display is required, set the image with which the display should start. ("0" is set when this button is not used.)

<Set end position> button

When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, "n-1" is set when this button is not used.)

<XYZ series> button

Displays one of multiple images by selecting it according to the multiple sections (Z).

<XYT series> button

Displays one of multiple images by selecting it according to the time (T).

<Bound> button

Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.

<Display> button

Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame-by-frame.

<Rabbit> button

Press for successive display of image slices without pause.

<Turtle> button

Press for successive display of image slices at an interval of 0.8 sec.

<Display> button

Press to start display of n-slice of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)

5. Display the image slice to end the successive display by using the <Display> button at the top of the image window.

6. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming that the number of images is n, becomes the start image automatically.)

7. Click the <Display> button. The images will be displayed successively from the start position to the end position.
   To stop the successive display, click the <Stop> button again.

2-11 Transferring Data to Another Application

2-11-1 Transferring Analysis Data to Another Application

Analysis data can be transferred to Excel.

1. Display the [Analyze] panel and executes analysis. After it, display the [Enhanced Profile Plot], [Intensity Map], [Enhanced Histogram Plot], {Average Intensity Trace] or [Integrated Intensity Trace].

TIP

For the operation method, see section 2-7, “Image Analysis”.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

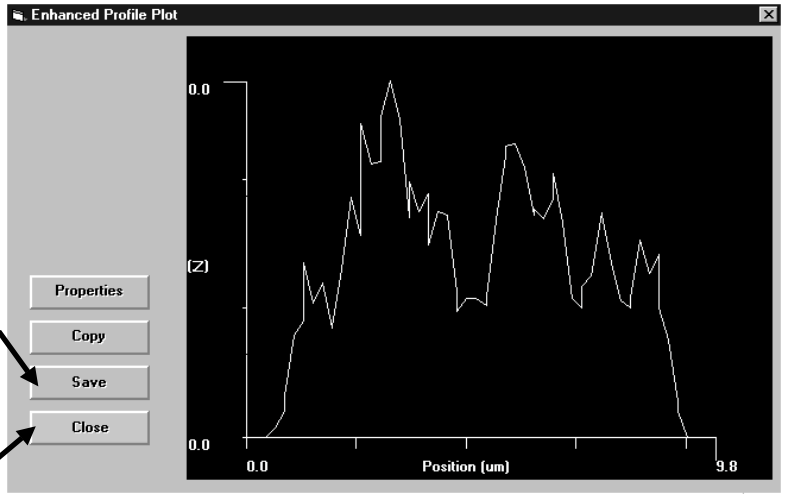


Fig. 2-132 [Enhanced Profile Plot] Window

TIP

Microsoft Excel is not included in the FLUOVIEW FV1000 system.

Please purchase it separately.

2. Click the <Save> button. When the [Save As] dialog box appears as shown below, set the file name and click the <OK> button to save the analysis data.

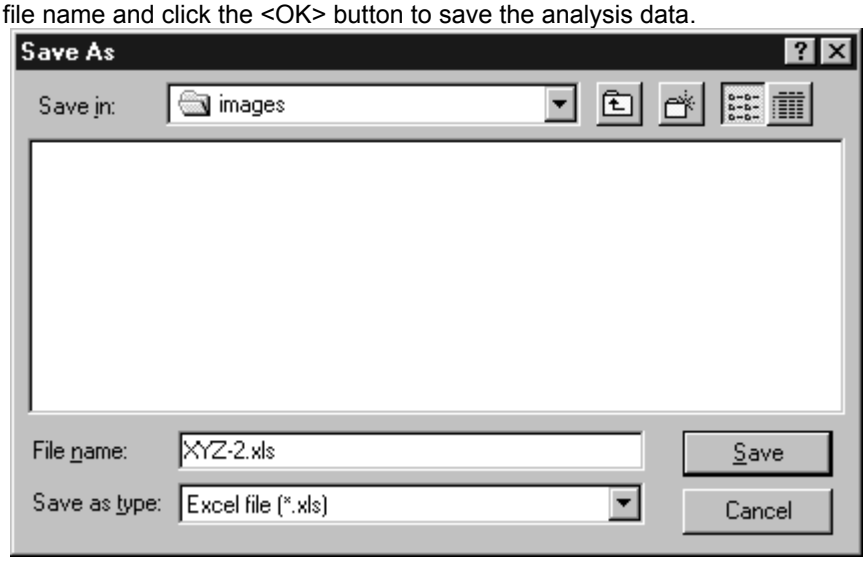


Fig. 2-133 [Save As] Dialog Box

3. Exit from FLUOVIEW or display the [Start] menu by pressing the Ctrl + Esc keys.
4. Select [Programs] and issue the [Microsoft Excel] command.
5. From the [File] menu of Excel, select the [Open] command and open the file saved in step 2.
6. When the dialog box as shown below appears, click the [Delimited] option button in the [Original Data Type] group box, then select [Windows (ANSI)] from the [File Origin:] drop-down list.

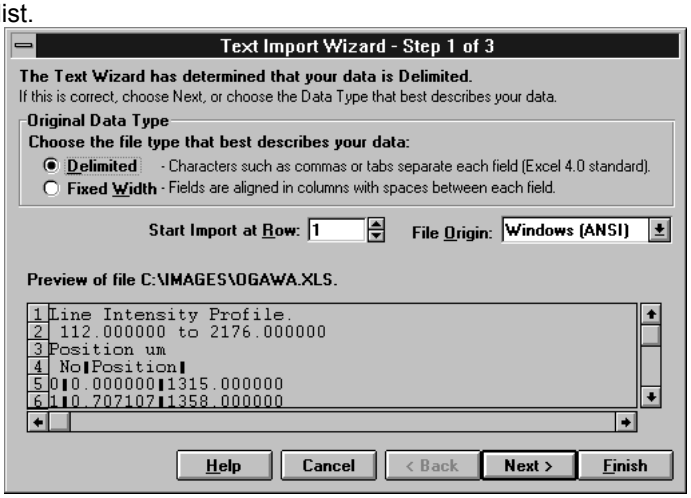

Text Import Wizard - Step 1 of 3
The Text Wizard has determined that your data is Delimited.
If this is correct, choose Next, or choose the Data Type that best describes your data.
Original Data Type
Choose the file type that best describes your data:
Delimited - Characters such as commas or tabs separate each field (Excel 4.0 standard).
Fixed Width - Fields are aligned in columns with spaces between each field.
Start Import at Row: 1
File Origin: Windows (ANSI)
Preview of file C:\IMAGES\OGAWA.XLS.
1 Line Intensity Profile.
2 112.000000 to 2176.000000
3 Position um
4 No Position
5 0 0.000000 1315.000000
6 1 0.707107 1358.000000
Help
Cancel
< Back
Next >
Finish

Fig. 2-134 Dialog Box When the File is Opened by Excel 1/3

7. Click the <Next> button. When the dialog as shown below appears, check the [Tab] check box in the [Delimiters] group box, then select [[none]] from the [Text Qualifier:] drop-down list.

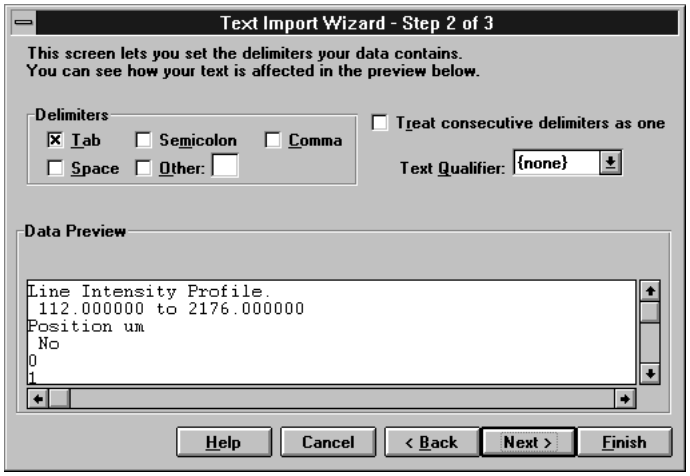


Fig. 2-135 Dialog Box When the File is Opened by Excel 2 / 3

8. Click the <Next> button. When the dialog as shown below appears, select the [General] option button in the [Column Data Format] group box, then click the <Finish> button.

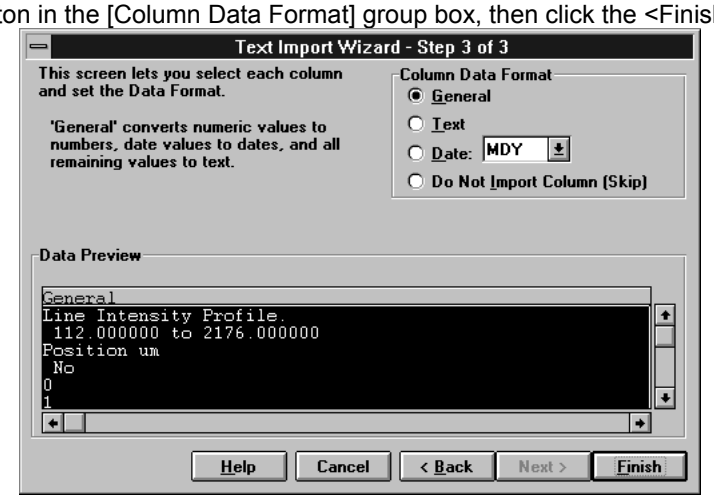


Fig. 2-136 Dialog Box When the File is Opened by Excel 3 / 3

TIP

For detailed operation procedures of Excel, refer to the [Excel manuals].

A1 Line Intensity Profile.

	A	B	C	D	E	F	G	H
1	Line Intensity Profile.							
2	112.000000 to 2176.000000							
3	Position um					0.000000 to 181.048776		
4	No	Position						
5	0	0	1315					
6	1	0.707107	1358					
7	2	1.414214	1353					
8	3	2.12132	1334					
9	4	2.62132	1365					
10	5	3.328427	1404					
11	6	4.035534	1276					

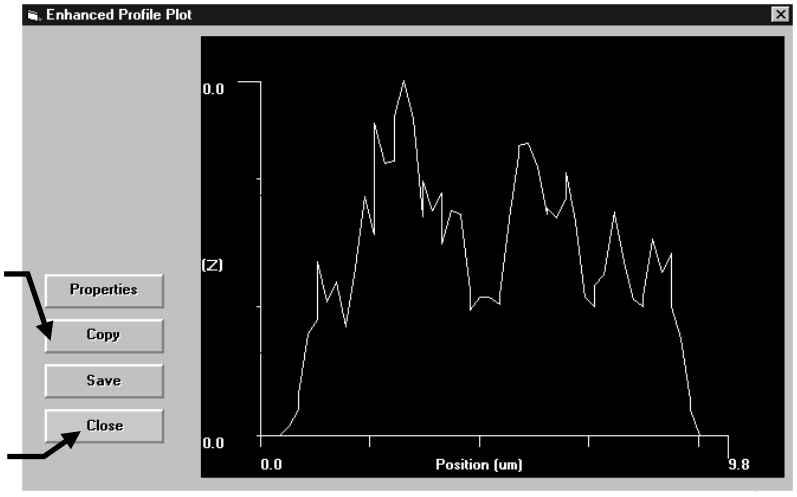
2-11-2 Transferring the Plot Image of Analysis Data to Another Application

The plot image of analysis data can be transferred to an application handling images, such as Paint. The following description takes Paint as example.

1. Display the [Analyze] panel and executes analysis. After it, display the [Enhanced Profile Plot], [Intensity Map], [Enhanced Histogram Plot], {Average Intensity Trace] or [Integrated Intensity Trace].

TIP

For the operation method, see section 2-7, “Image Analysis”.



<Copy> button
Copies the plot image to the clipboard.

<Close> button
Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

Fig. 2-137 [Enhanced Profile Plot] Window

2. Click the <Copy> button to copy the plot image to the clipboard.
3. Exit from FLUOVIEW or display the [Start] menu by pressing the Windows key or the Ctrl + Esc keys.
4. Select [Programs]-[Accessories] and issue the [Paint] command.
5. From the [Edit] menu of Paint, select the [Paste] command and paste the plot image which has been copied to the clipboard in step 3.

TIP For detailed operation procedures of Paint, refer to the [help provided by Paint].

2-11-3 Transferring Image Data to Another Application (Paint, etc.)

To transfer image data to another application, the image data should be saved in a file and the file should be transferred.

1. Save an image using one of the formats that can be handled by the destination application. See sections 2-3, “Saving, Opening and Shredding Images” and 2-3-1-1, “Saving Images As a Series” for the image saving procedure.

2. Exit from FLUOVIEW or display the [Start] menu by pressing the Windows key or the Ctrl + Esc keys.

3. Start the image transfer destination application (Paint, etc.).

4. In the application, open the file saved in step 1.

2-12 Entering Comment in Image

Comment can be entered in an image for use in presentation or slide creation.

<Annotate> button

Use the <Annotate> button in the toolbar. Click the <Annotate> button. A list of buttons appears as shown below.

2-12-1 Writing Characters in Image

This facility is used to enter the title, acquisition parameters and/or notes in an image. Some labels are provided in advance. Characters can be written either by using these labels or entering desired characters at will.

<Text> button

1. Display the image window of the image in which you want to write characters.
2. Click the <Text> button in the displayed list of buttons. The dialog as shown below appears.

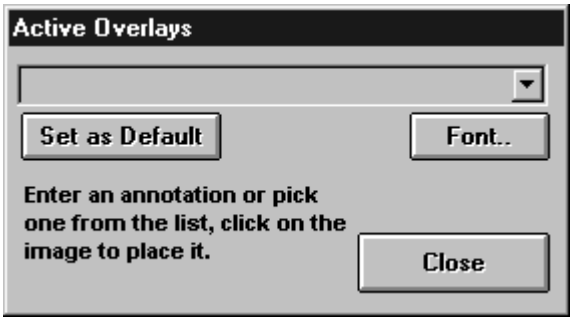


Fig. 2-138 [Active Overlays] Dialog Box

3. From the drop-down list in the dialog box, select one of the labels.

TIP

Desired characters can also be entered.

1. Click the character in the drop-down list of the dialog box.
2. Delete the character by pressing the [Delete] or [Back Space] key
3. Enter characters from the keyboard.

TIP

When the <Set as Default> button is pressed after having selected a label, it is set as the label displayed permanently at the bottom left of the image window.

4. Click the <Font> button. The dialog box as shown below appears.

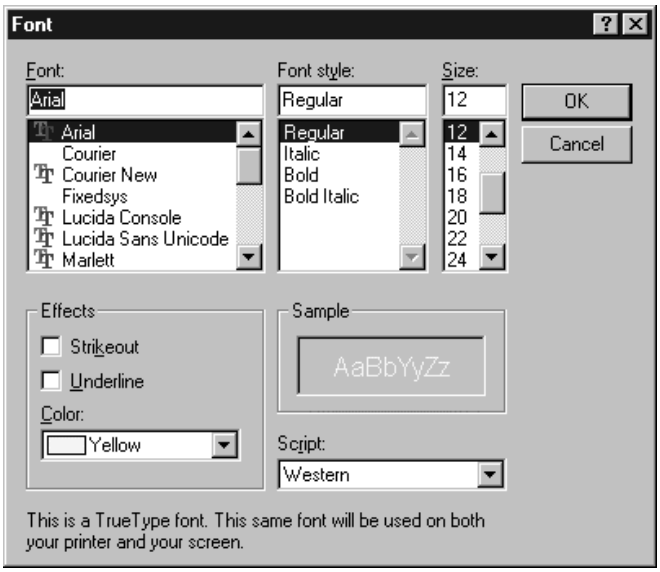


Fig. 2-139 [Font] Dialog Box

5. Select the character font and size using the [Font], [Font Style] and [Size] list boxes.
6. Click the <OK> button to close the [Font] dialog box.
7. Place and click the mouse pointer on the image position you want to enter characters.

TIP

Refer to “Appendix J List of Functions in the [Active Overlays] Dialog Box” for other functions of the [Active Overlays] dialog box.

2-12-2 Displaying the Image Intensity

The intensity of any pixel of an image can be displayed without using the [Analyze] panel.

1. Display the image window of the image that you want to display the intensity at the front.

<Text> button

2. In the list of buttons displayed, click the <Text> button. The dialog box as shown below appears.

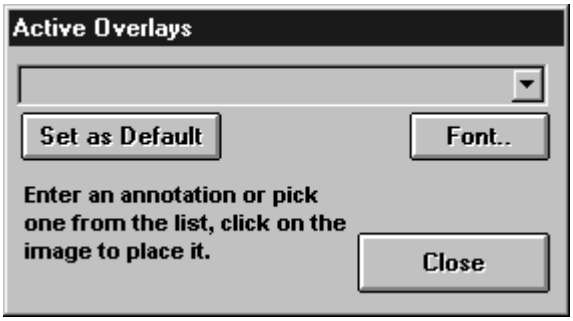


Fig 2-140 [Active Overlays] Dialog Box

3. In the drop-down list inside the dialog box, select" I = <intensity hotspot value>".

4. On the image, place the mouse pointer on the position that you want to display its intensity and click the mouse.

2-12-3 Displaying the X-coordinate/Y-coordinate of the Image

The X-coordinate position or the Y-coordinate position of any pixel of an image can be displayed.

<Text> button

1. Display the image window of the image that you want to display the X-coordinate position or the Y-coordinate position at the front.

2. In the list of buttons displayed, click the <Text> button. The dialog box as shown below appears.

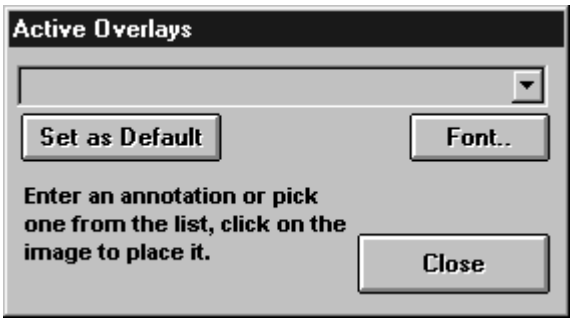


Fig 2-141 [Active Overlays] Dialog Box

3. In the drop-down list inside the dialog box, select “x = <x hotspot value “” units> “ to display the X-coordinate position or “Y = hotspot value “” units>“ to display the Y-coordinate position.

4. On the image, place the mouse pointer on the position that you want to display the X-coordinate position or the Y-coordinate position and click the mouse.

TIP

The displayed value indicates the distance from the origin when assuming the upper left of the image as the origin.

2-12-4 Drawing a Figure in Image

This facility is used to draw figures in the image. FLUOVIEW provides seven figure drawing modes.

1. Display the image window of the image in which you want to draw figures.
2. Select one of the following command buttons and draw a figure in the image using the mouse. The operation methods of the command buttons are described below.

<Line> button

To draw a straight line:

On the image, click the point that you want to start the straight line, and drag the mouse from there to the point you want to end it.

<Poly Line> button

To draw a polygonal line:

On the image, click the points corresponding to the start point, peak points and end point of the desired polygonal line, then click the right button of the mouse to set the specification.

<Rectangular> button

To draw a rectangle:

On the image, drag the mouse pointer along the diagonal line of the desired rectangle, from the top left corner to the bottom right corner.

<Circle> button

To specify a circle or ellipse:

On the image, assume a rectangle circumscribing the circle to be checked and drag the mouse pointer along the diagonal line between opposite corners of the rectangle.

<Polyregion> button

To specify a polygonal region:

On the image, click the points corresponding to the corners of the desired polygon. After clicking the last corner point, click the right button of the mouse to connect the last clicked point to the first clicked point.

<Free Region> button

To specify a free region:

On the image, specify a region by dragging. Then release the mouse button to complete dragging. The point where the dragging was ended will be connected to the point where it was started.

<Free Line> button

To specify a free line:

On the image, drag the mouse pointer along the desired line.

2-12-5 Drawing a Scale in Image

A scale can be drawn between two points in an image.

<Scale> button

1. Display the image window of the image in which you want to draw a scale.
2. Click the <Scale> button in the displayed list of buttons.
3. Click the image position you want to draw a scale.
4. Change the scale size. See 2-12-8, “Changing the Comment Size” for the operation procedure.

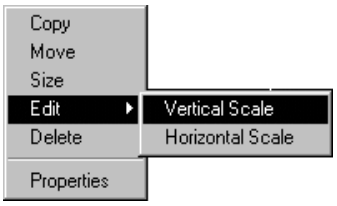
NOTE

To display a correct scale, it is required that the image has been acquired while the objective magnification setting in the software matches the magnification of the actually used objective.

TIP

A vertical scale can be drawn as well as a horizontal scale.

1. Click the mouse on the scale to turn the scale active (i.e. handles displayed around the scale).
2. Click the right mouse button.
3. Select [Edit] from the displayed menu. A sub-menu as shown below appears.



4. Select [Vertical Scale] to display a vertical scale.

TIP

The size of characters in the scale can also be changed.

1. Click the mouse on the scale. In the pop-up menu displayed, select [Properties].
2. Set the scale color on [Color] tab in [Properties] dialog box and the font on [Font] tab
3. Select the <OK> button to close the [Properties] dialog box.

2-12-6 Drawing an Arrow in Image

This facility is used to draw an arrow for indicating a point in interest in image or adding explanation in it.

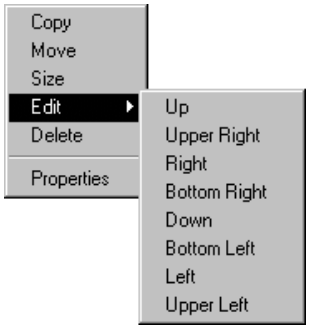
<Arrow> button

1. Display the image window of the image you want to draw an arrow.
2. Click the <Arrow> button in the displayed list of buttons.
3. Drag the mouse pointer from the start point to the end point of the desired arrow.
4. To change the arrow size, see section 2-12-8, “Changing the Comment Size”.

TIP

The direction indicated by a previously drawn arrow can be changed.

1. Click the mouse on the arrow to make the arrow active (i.e. handles displayed on the arrow).
2. Click the right button of the mouse.
3. Select [Edit] from the displayed menu. A sub-menu as shown below appears.



4. Select the desired arrow direction from the sub-menu.

2-12-7 Drawing Color Bars in Image

This facility is used to draw color bars in an image.

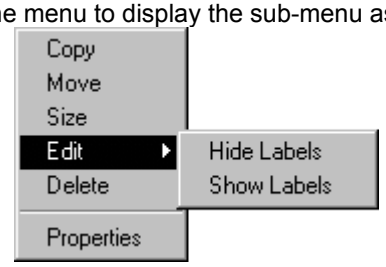
<Color Bar> button

1. Display the image window of the image you want to draw color bars.
2. Click the <Color Bar> button in the displayed list of buttons.
3. Draw color bars in the image by dragging the mouse pointer along the desired position in the image.
4. To change the color bar size, see section 2-12-8, “Changing the Comment Size”.

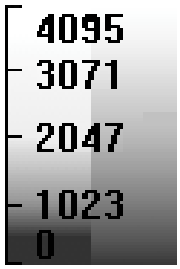
TIP

The labels of the color bars can be switched to display or hide.

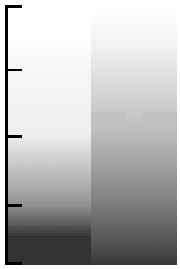
1. Click the mouse on/inside the color bars to select. (The handles appear arround the color bars.)
2. Right-click the mouse.
3. Select [Edit] in the menu to display the sub-menu as shown below.



4. In the sub-menu, select “Show Labels” to display the labels or “Hide Labels” to hide the labels.

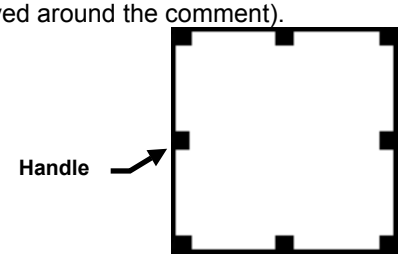


The labels are shown


The labels are hidden

2-12-8 Deleting Comment

1. Click the mouse on the comment to be deleted to make the comment active (i.e. handles displayed around the comment).



2. Click the right button of the mouse. A pop-up menu as shown below appears.

Fig. 2-142 Pop-up Menu

3. Select [Delete] from the menu.

TIP

To delete more than one comment simultaneously:

Select multiple comments and select [Delete] from the pop-up menu.

To select, click the mouse on or in the middle of one of the comments and click the moue while holding the Shift key to select the second comment and after.

After making all of the comments to be deleted active (i.e. handled displayed around them), click the right button of the mouse on one of the comments and select [Delete] from the displayed pop-up menu.

2-12-9 Moving Comment

1. Click the moues on the comment to be moved to make it active (i.e. handles displayed around it).

2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-142 appears.

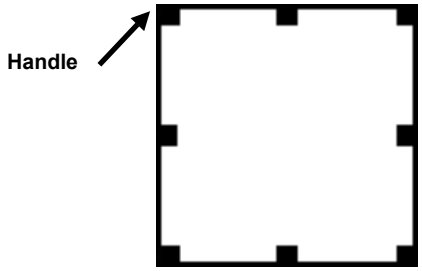
3. Select [Move] from the menu.

4. The mouse pointer turns into a cross (+). Move the mouse to move the comment together with the mouse pointer.

5. Click the left button of the mouse to determine the new position.

TIP

A comment can also be moved by selecting it, placing the mouse pointer on it so that the mouse pointer turns into a cross (+), then dragging the mouse. In this case, the final positioning of the comment can be determined by placing the mouse pointer outside the areas enclosed by the handles and clicking the left button of the mouse.

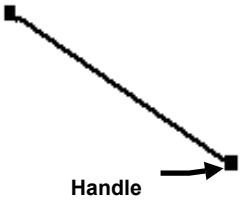


NOTE

The mouse pointer may be hardly visible depending on the image being displayed. In this case, use the method of displaying the pop-up menu.

2-12-10 Changing the Comment Size

1. Click the mouse on the comment to be resized to make it active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-142 appears.
3. Select [Size] from the menu.
4. The menu pointer turns into ↕, When the mouse is moved, the comment is magnified or reduced according to the mouse pointer.
5. Click the left button of the mouse to determine the size.



TIP

A comment can also be magnified or reduced by selecting it, placing the mouse pointer on one of the handles so that the mouse pointer turns into ↕, then dragging the mouse.

2-12-11 Changing the Comment Color

1. Click the mouse on the comment to be changed of color to make the comment active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-142 appears.
3. Select [Properties] from the menu. The [Properties] dialog box as shown below appears. Display the [Color] panel at the front.

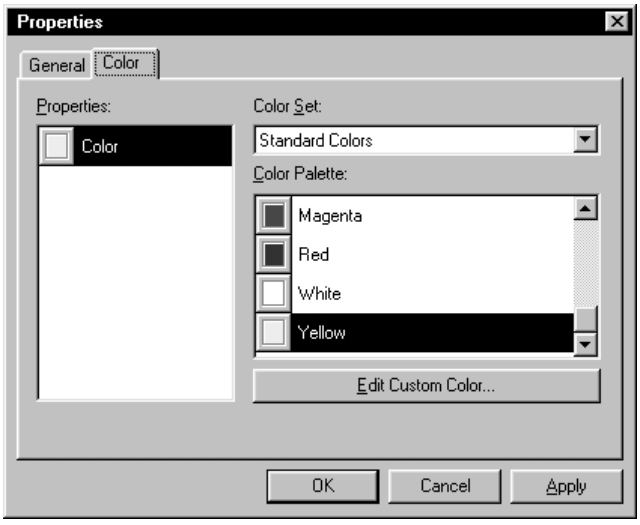


Fig. 2-143 [Properties] Dialog Box

4. Select the desired color from the [Color Palette] list box.

TIP

To change the comment color automatically, set in the [FLUOVIEW Setup] dialog box.

For details, see the description on the [Software] panel in section 1-3-1 in MAINTENANCE, “Overall Setting of FLUOVIEW” for detailed operations.

2-12-12 Changing the Comment Font

1. Click the mouse on the comment to be changed of font to make the comment active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-142 appears.
3. Select [Properties] from the menu. The [Properties] dialog box as shown below appears. Display the [Font] panel at the front.

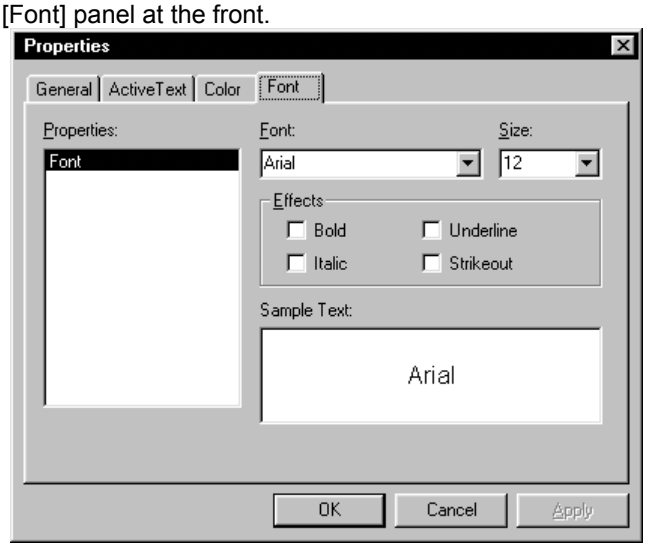


Fig 2-144 [Properties] Dialog Box

4. Set the font type and size using the drop-down lists and select the effect.

2-13 Image Output at Printer

<Print> button

1. Display the image window of the image to be output at the printer.
2. Click the <Print> button in the toolbar at the bottom left of the panel. A dialog box as shown below appears.

Fig. 2-145 [Print] Dialog Box

3. Select the connected printer name from the [Name] drop-down list.
4. If it is required to set the detailed data of the printer, click the <Properties> button in the dialog box.
5. Click the <OK> button of the dialog box.

NOTE

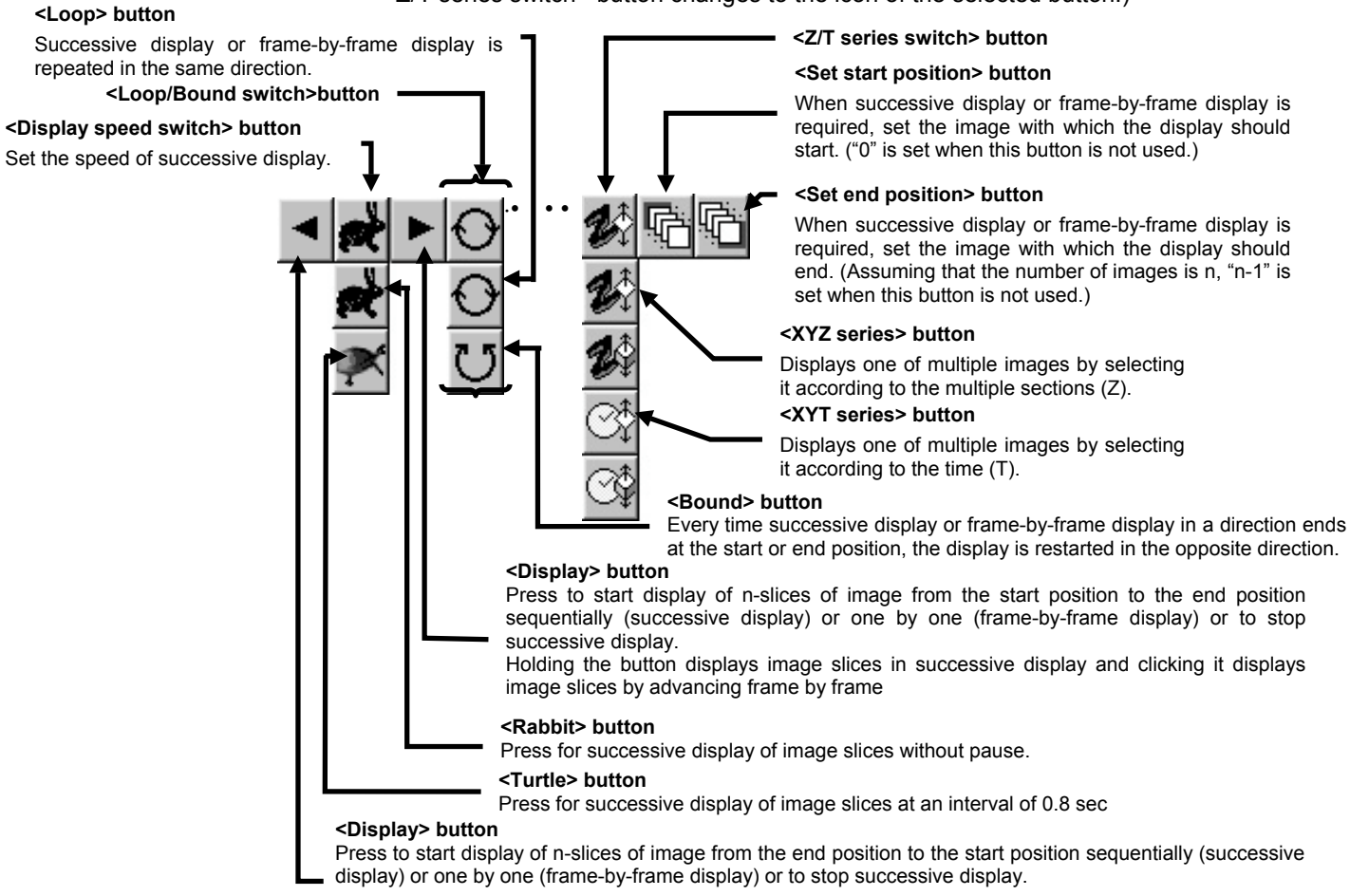
It is required to install and select the printer driver before the above operation. Refer to the Windows manuals for details.
For the printer operation procedures, refer to the printer manuals.

2-14 Merger/Extraction of Image Channels

2-14-1 Setting the Range of Multiple Image Slices

When merging or extracting channels in images composed of multiple image slices, such as time-lapse images and images acquired by varying the cross-sections, it is possible to select only some of image slices as the target of channel merger or extraction. This section describes how to set the range of target image slices.

1. Display the image window of the images composed of multiple image slices.
2. The buttons as shown below are displayed at the top of the image window. To switch to the image slice of another cross-section, click the <Z/T series switch> button then click the <XYZ series> button in the displayed list of buttons. To switch to the image slice of another instant in the elapsed time, click the <Z/T series switch> button then click the <XYT series> button in the displayed list of buttons. (In these operations, the icon in the <Z/T series switch> button changes to the icon of the selected button.)


<Loop> button
Successive display or frame-by-frame display is repeated in the same direction.
<Loop/Bound switch>button
<Display speed switch> button
Set the speed of successive display.
<Z/T series switch> button
<Set start position> button
When successive display or frame-by-frame display is required, set the image with which the display should start. ("0" is set when this button is not used.)
<Set end position> button
When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, "n-1" is set when this button is not used.)
<XYZ series> button
Displays one of multiple images by selecting it according to the multiple sections (Z).
<XYT series> button
Displays one of multiple images by selecting it according to the time (T).
<Bound> button
Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.
<Display> button
Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame
<Rabbit> button
Press for successive display of image slices without pause.
<Turtle> button
Press for successive display of image slices at an interval of 0.8 sec
<Display> button
Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

3. Display the image slice to start the range at the front using the <Display> button at the top of the image window.
4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
5. Display the image slice to end the range at the front using the <Display> button.
6. Click the <Display> button. (If the end position is not set, image No. n-1, assuming the number of image slices is n, becomes the start image automatically.)

2-14-2 Merging Image Channels

For instance, when a specimen is imaged with two scans for 2-channel fluorescence observation and 1-channel transmitted light observation, the transmitted light image can be merged to the fluorescence images to create a 3-channel image. Note that the display gradation of the images obtained by overlaying 3 channels may be poor than original.

1. Open the two files to be used in creating a new image.

NOTE

The image files used in overlay are subjected to the following restrictions.

· The sizes of the images in the two image files should be identical.

· The number of data bits in the two image files should be identical.

(For example, it is not possible to overlay a Fluoview Multi Tiff file with a Single TIF(S) 8-bit file.)

TIP

When the image in one of the image files is composed of multiple image slices, it is possible to use only some of the slices by setting a slice range. See section 2-14-1, “Setting the Range of Multiple Image Slices” for the operation procedure.

When the images in both image files are composed of multiple image slices, the overlaid image will have the same number of image slices as the file with the fewer image slices.

Example 1: XYZ image + XY image = XY image

(The first image slice of the XYZ image is overlaid with the XY image.)

Example 2: XYZ image with 10 slices + XYZ image with 5 slices = XYZ image with 5 slices

(The first five image slices are overlaid.)

2. Display the [Experiment Editor] sub-panel in the [Process] panel.

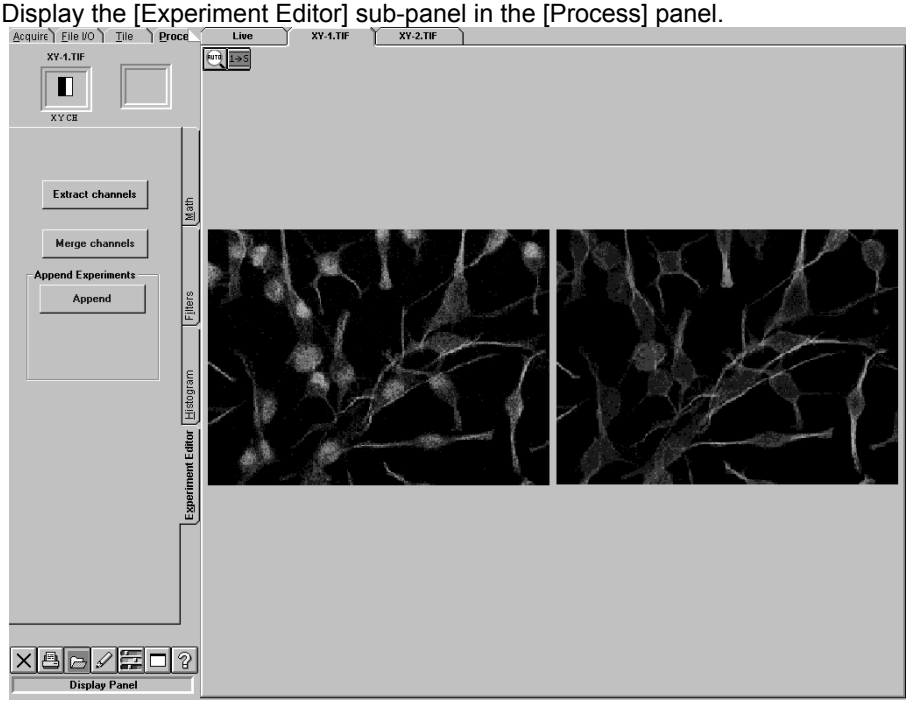


Fig. 2-146 [Experiment Editor] Sub-panel

3. The [Experiments in Memory] dialog box appears as shown below.

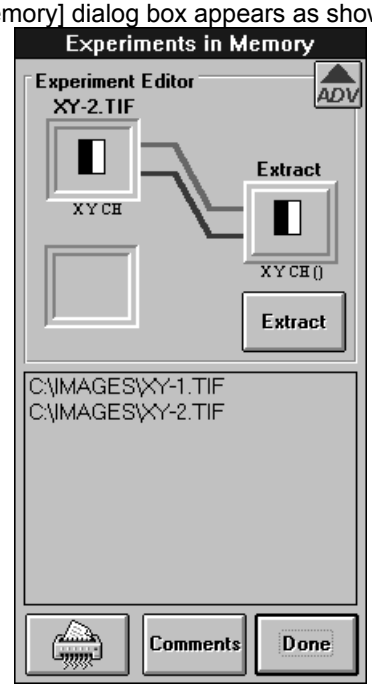
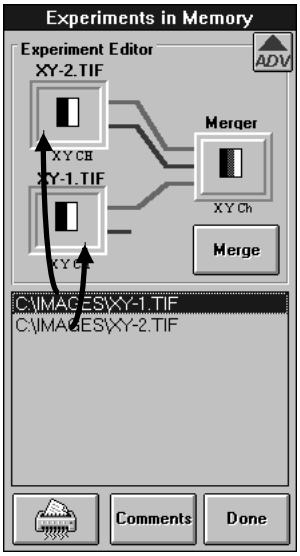


Fig. 2-147 [Experiment Editor] Group Box

TIP The frame at the top left of the [Experiments in Memory] dialog box shows the icon of the image file displayed at the front of the image window.



4. From the file list in the [Experiments in Memory] dialog box, select the file name of the first image and drag it into the frame at the top left. The icon of the first image is displayed in the frame at the top left.

TIP The mouse pointer turns into the image icon during dragging.

5. From the file list in the [Experiments in Memory] dialog box, select the file name of the second image and drag it into the frame at the top left. The icon of the second image is displayed in the frame at the top left of the [Experiments in Memory] dialog box.

TIP The mouse pointer turns into the image icon during dragging.

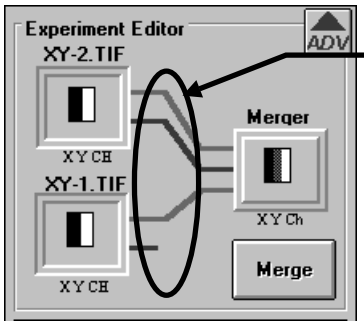
NOTE **When the image of the second selected image file is composed of multiple image slices, the setting of the image slice range is ineffective even when the range is set.**

6. Up to 3 channels are selected automatically beginning with the first image set in the [Experiments in Memory] dialog box, and connected to [Merger] by lines.

TIP The lines from the image to [Merger] represent the channels, which are drawn in order of numbers from the top to the bottom.

TIP

When the mouse pointer is approached to the icon-side end of a line connected to [Merger], the color of the line end turns into yellow. Clicking the line in this condition switches the channel between the selected and deselected status alternately. The lines connected to [Merger] indicate the selected channels.



Clicking a channel line in this area switches the channel between the selected and deselected status.

NOTE

Up to 6 channels can be selected together.

To select another channel after having selected 6 channels, deselect the unnecessary channels before selecting required channels.

7. Click the <Merge> button in the [Experiments in Memory] dialog box. A new image window showing [Merge] in the page tab appears and the images including newly merged channel(s) are displayed in the panel.

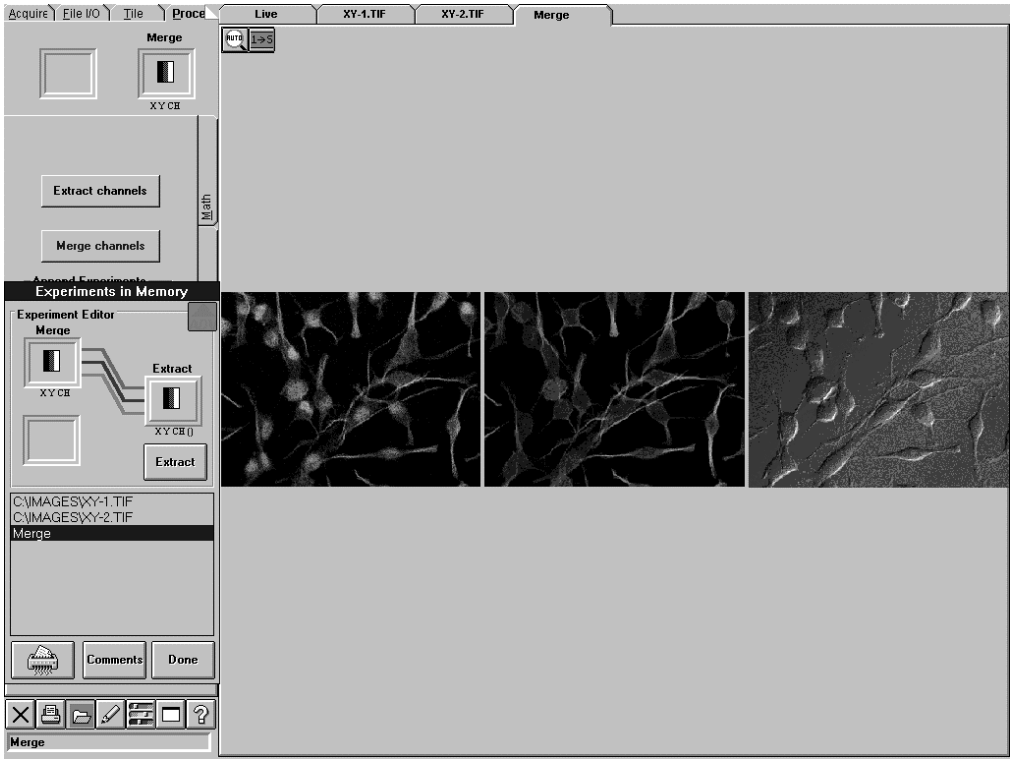


Fig. 2-148 [Merge] Panel

2-14-3 Extracting Channels from Image

Desired channels can be extracted from an image. Use this facility to extract only the images of required channels from an image acquired from more than one channel.

1. Open the image file of the image to extract channels in advance.

TIP

When the image has been saved in more than one image file, it is possible to use only some of the slices by setting a slice range. See section 2-14-1, “Setting the Range of Multiple Image Slices” for the operation procedure.

NOTE

The channel extraction cannot change the image type (XYZ, XYT, etc.). Therefore, it is not possible, for example, to extract an XY image from an XYZ or XYT image.

2. Display the [Experiment Editor] sub-panel in the [Process] panel.

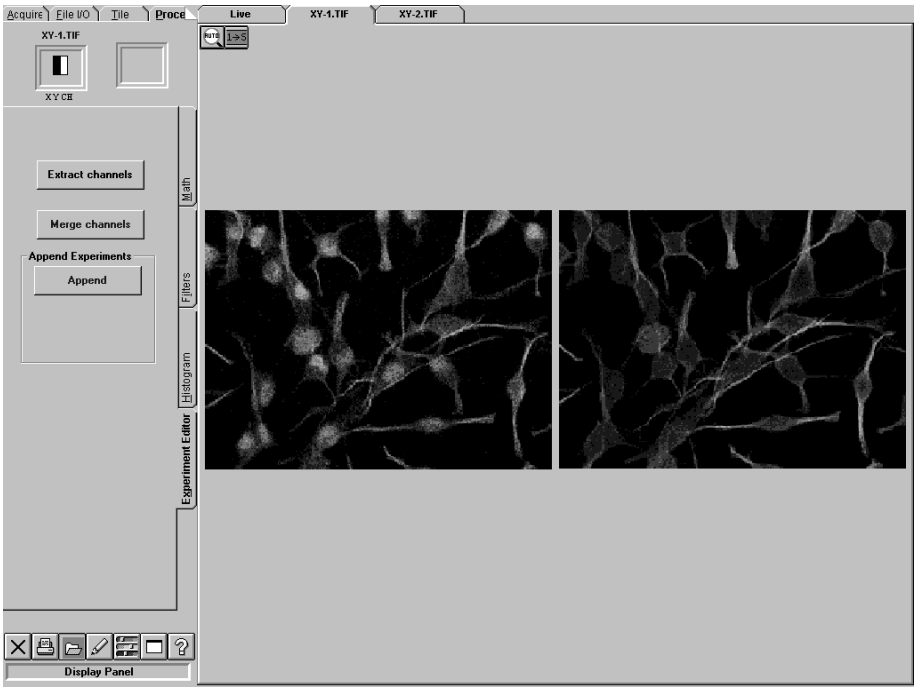


Fig. 2-149 [Experiment Editor] Sub-panel

3. The [Experiments in Memory] dialog box appears as shown below.

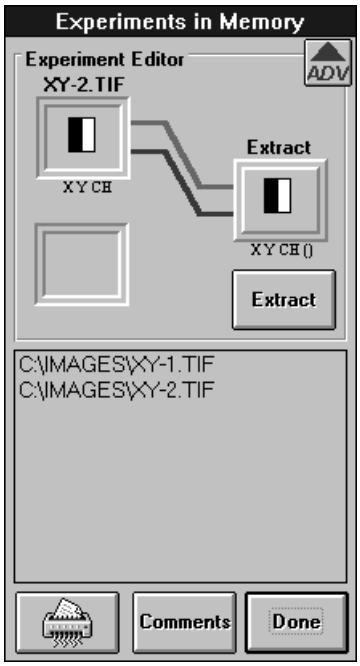
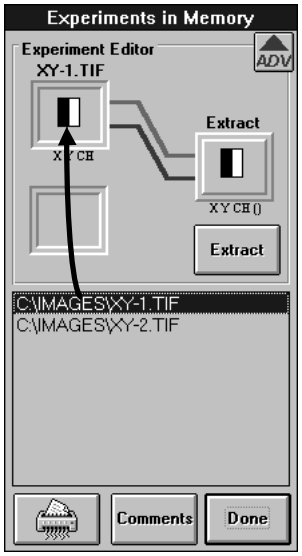


Fig. 2-150 [Experiment Editor] Group Box

TIP The frame at the top left of the [Experiments in Memory] dialog box shows the icon of the image file displayed at the front of the image window.



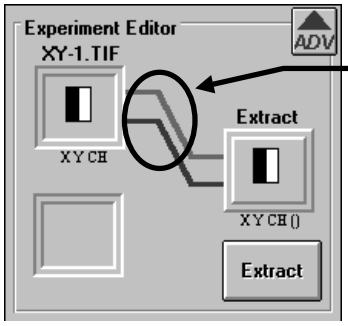
4. From the file list in the [Experiments in Memory] dialog box, select the file name of the image from which to extract channels and drag it into the frame at the top left. The icon of the image is displayed in the frame at the top left.

TIP The mouse pointer turns into the image icon during dragging.

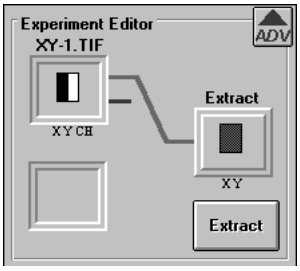
5. The channels of the image set in the [Experiments in Memory] dialog box are connected to [Extract] by lines.

TIP The lines from the image to [Extract] represent the channels, which are drawn in order of numbers from the top to the bottom.

TIP When the mouse pointer is approached to the icon-side end of a line connected to [Extract], the color of the line end turns into yellow. Clicking the line in this condition switches the channel between the selected and deselected status alternately. The lines connected to [Extract] indicate the selected channels.



Clicking a channel line in this area switches the channel between the selected and deselected status.



6. Among the channel lines connected to [Extract], deselect the unnecessary channels as described in the TIP on the previous page so that only the necessary channels are selected.

7. Click the <Extract> button in the [Experiments in Memory] dialog box. A new image window showing [Extract] in the page tab appears and the image of the extracted channel(s) is displayed in the panel.

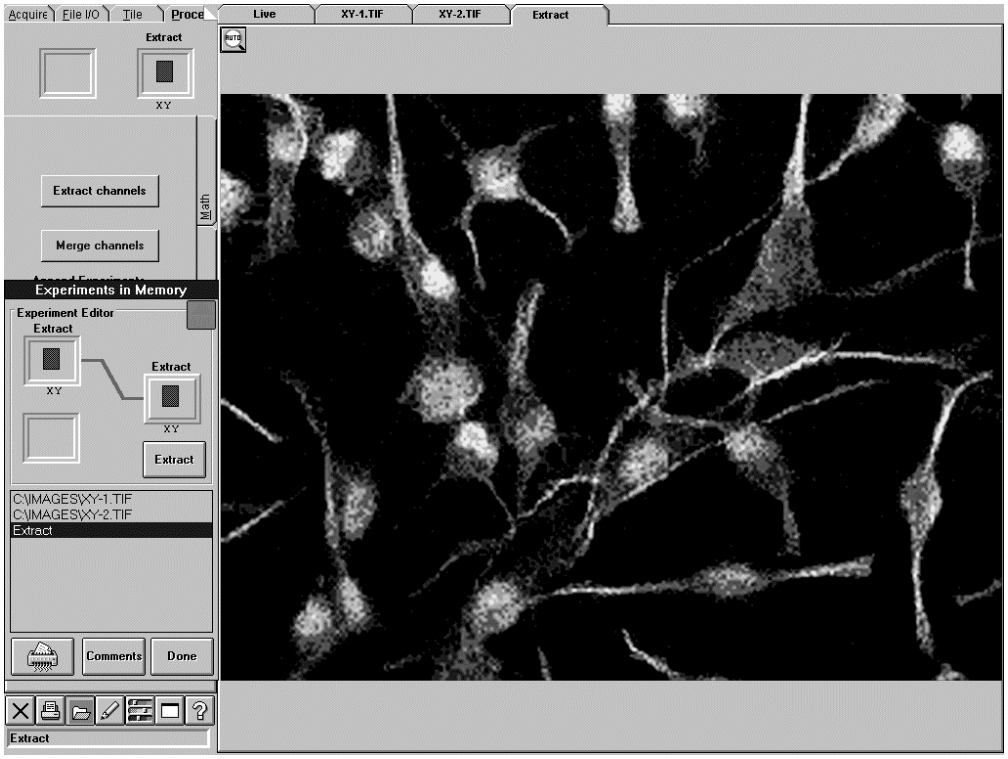


Fig. 2-151 [Extract] Panel

2-15 Changing the Chart Display Method

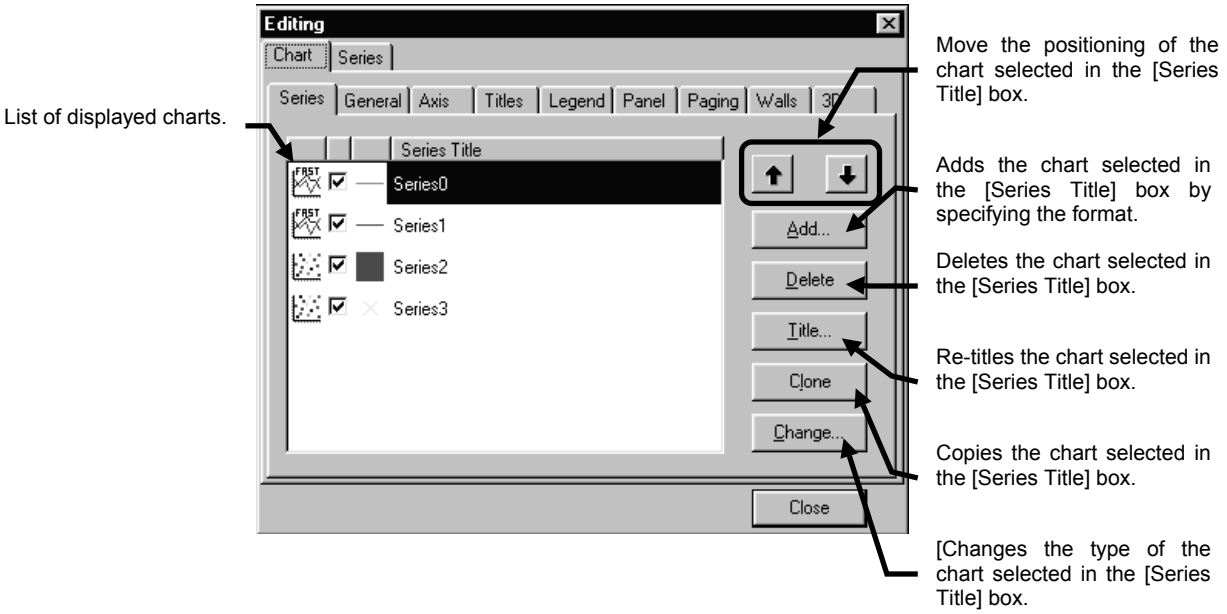
When the processed analysis data chart in the [Analyze] panel is double-clicked, the [Enhanced Profile Plot] dialog box appears.

By clicking the <Properties> button, the [Editing] dialog box can be displayed, allowing the detailed chart settings and chart display method to be changed.

2-15-1 [Chart] Panel

- [Series] panel

  This panel is used to change the type, title or other settings of the displayed chart as well as to add or delete charts.

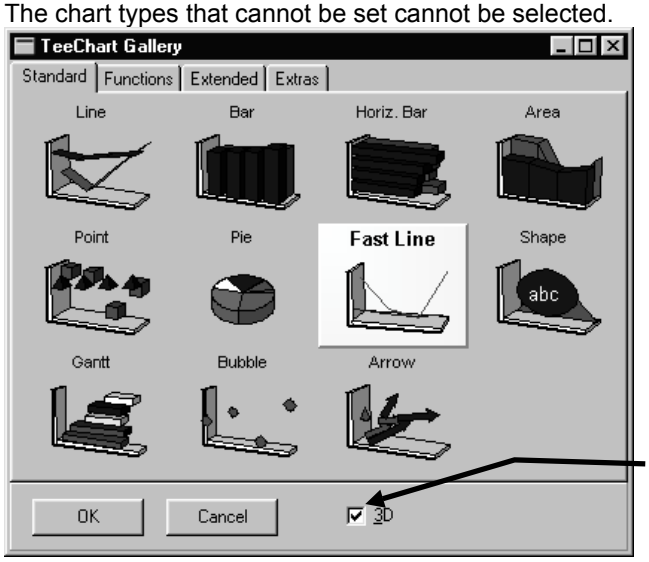


- [TeeChart Gallery] sub-panel

  Displayed when the <Add> or <Change> button is clicked.

  Used to set the chart type.

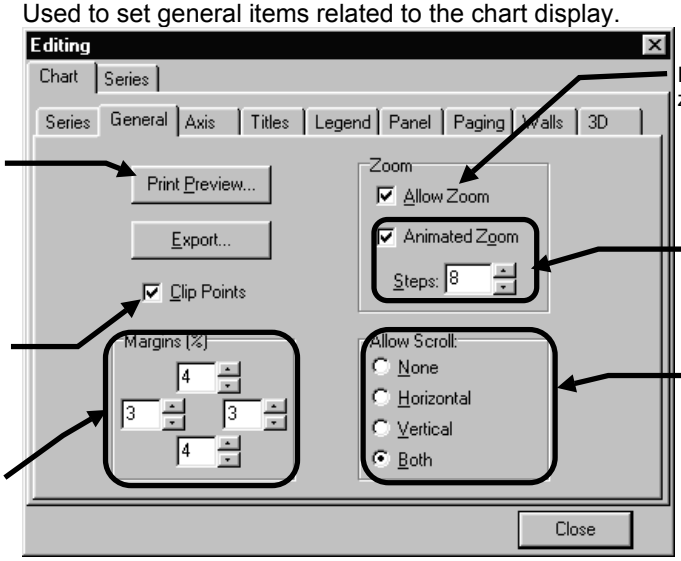
  The chart types that cannot be set cannot be selected.



Enables or disables the 3D display.

- [General] sub-panel

  Used to set general items related to the chart display.



Previews the printed chart or sets the orientation and margins of the print paper.

When a chart is displayed by zooming or scrolling, selects whether the data of only the area contained in the chart axes is to be displayed or not.

Sets the margins from the edges of the area in which the chart is displayed.

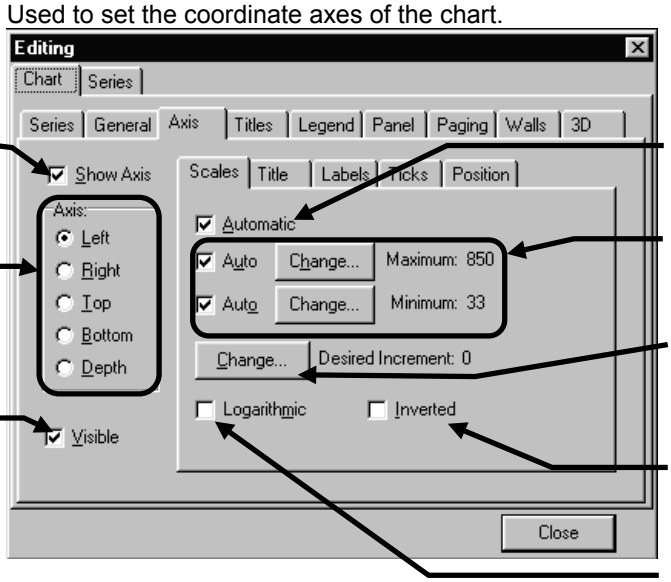
Enables or disables zooming.

When zooming is enabled, enables or disables gradual zooming according to the step count set in the [Steps:] dialog box.

Sets the chart scrolling direction.

- [Axis] sub-panel

Used to set the coordinate axes of the chart.



Enables or disables the display of chart axes.

Selects the chart axes to be set in the [Scales], [Title], [Labels], [Ticks] and [Position] panels.

Enables or disables the chart axis display set in the [Axis:] group box.

Displays the chart axes by selecting the optimum scale automatically.

Sets the maximum and minimum values of the chart axes either automatically or manually.

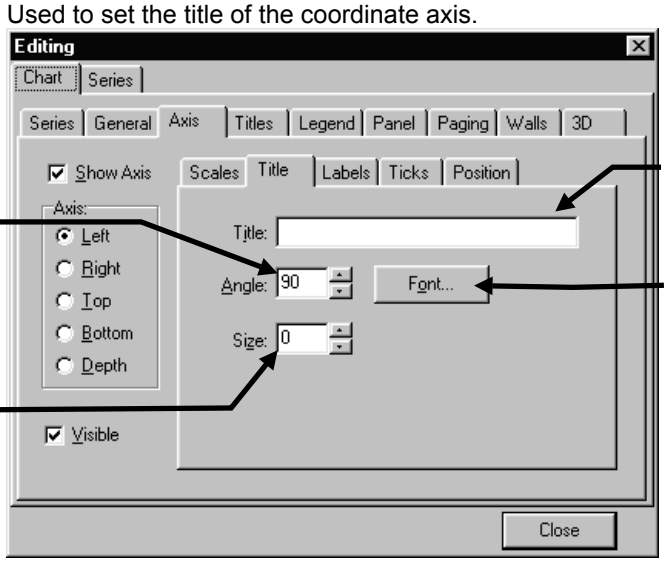
Sets the number of steps of the values displayed in the chart axis labels.

Enables or disables the inverted chart display.

Enables or disables the logarithmic chart display.

- [Title] sub-panel

Used to set the title of the coordinate axis.



Sets the angle of displaying the coordinate axis title.

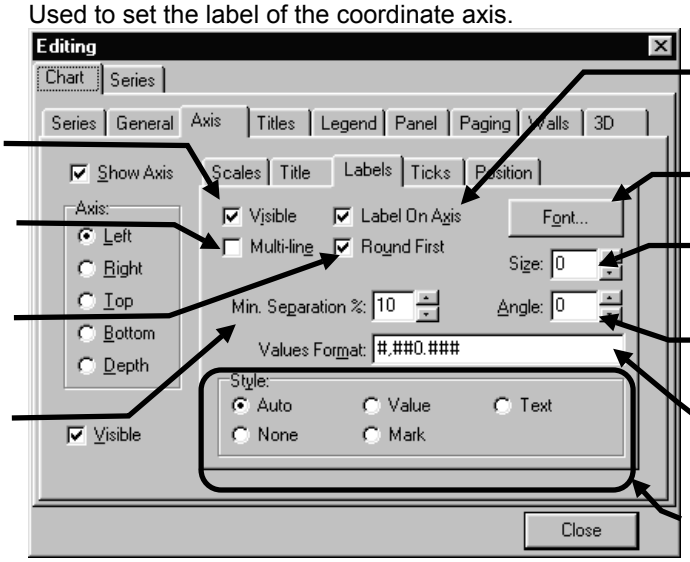
Sets the angle of displaying the coordinate axis title.

Sets the title of the coordinate axis.

Sets the title character font.

- [Labels] sub-panel

Used to set the label of the coordinate axis.



Enables or disables the label display.

Not used with the present application.

Enables or disables the scale display according to the maximum and minimum values in the chart.

Sets the distance between labels.

Enables or disables the label display on a point of crossing with another axis.

Sets the label character font.

Sets the distance from the title to the label display position.

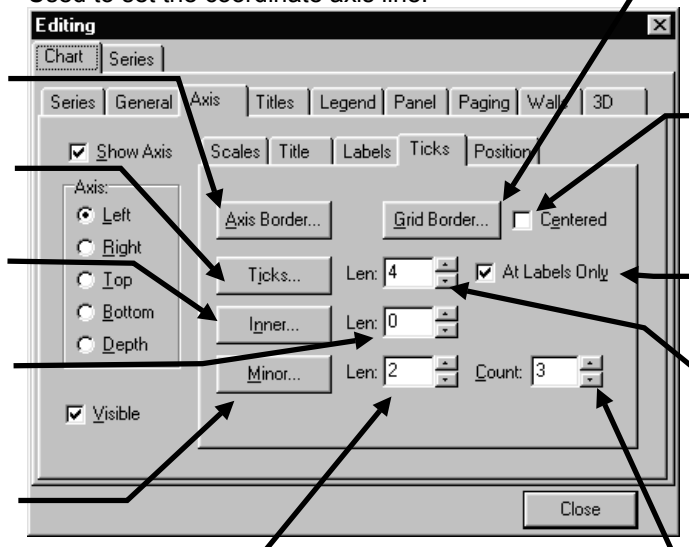
Sets the angle of label display.

Sets the label display format.

Sets the label type. Select [Auto] or [None].

- [Ticks] sub-panel

Used to set the coordinate axis line.



Sets the type, color and width of the coordinate axis.

Sets the type, color and width of the scale line on the label side of the coordinate axis.

Sets the type, color and width of the scale line on the chart side of the coordinate axis.

Sets the length of the scale line from the coordinate axis to the chart side.

Sets the type, color and width of the scale line between labels.

Sets the length from the coordinate axis of the scale line between labels to that on the label side.

Sets the type, color and width of the line along the label.

Enables or disables the display of coordinate axis scales in the intermediate position between labels.

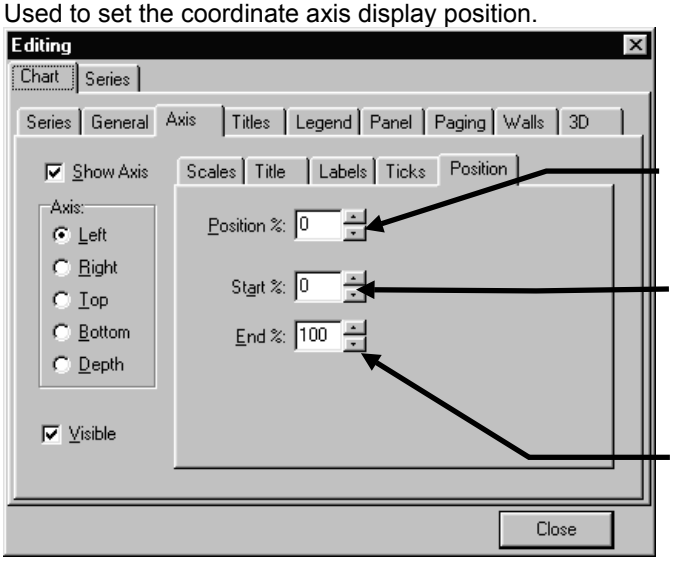
Enables or disables the display of scale line in the positions where labels are displayed.

Sets the length of the scale line from the coordinate axis to the label.

Sets the number of division between labels.

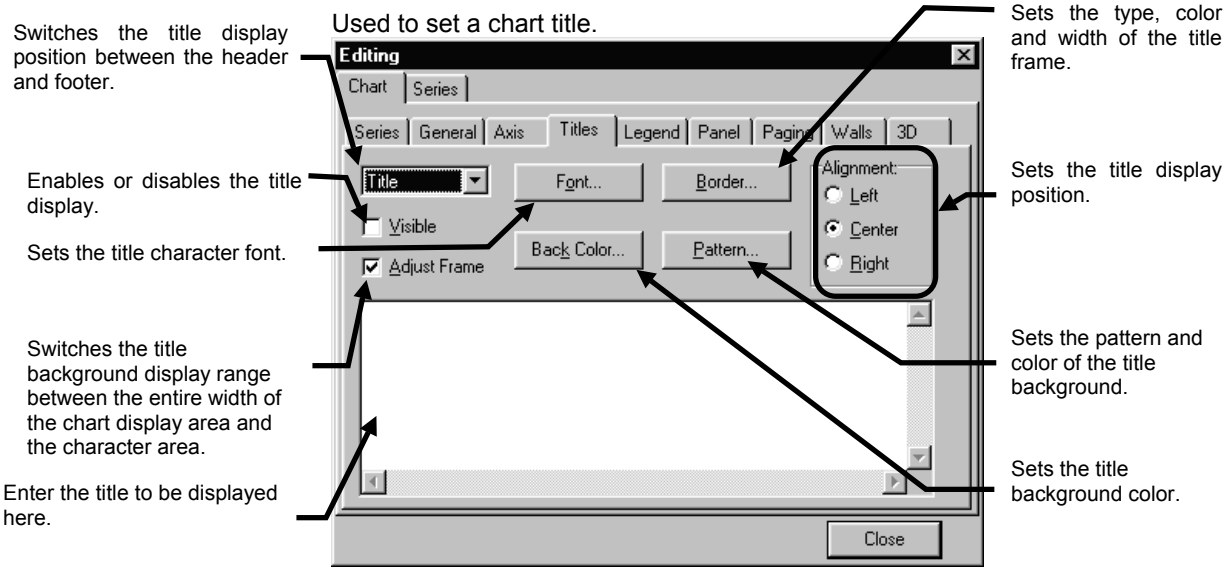
- [Position] sub-panel

Used to set the coordinate axis display position.



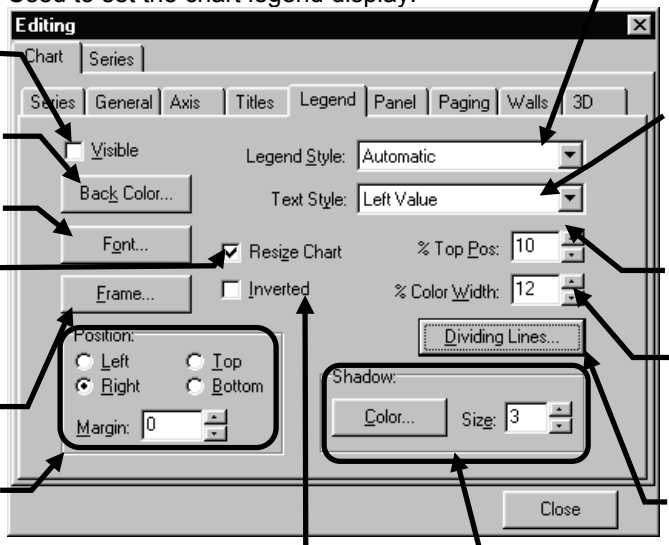
- [Titles] sub-panel

Used to set a chart title.



- [Legend] sub-panel

Used to set the chart legend display.



Enables or disables the legend display.

Sets the legend background color.

Sets the legend character font.

Sets whether or not the chart is resized according to the legend display.

Sets the type, color and width of the legend frame line.

Sets the legend display position and the margin between the chart display area and legend display area.

Sets the items to be displayed in the legend.
Usually set [Automatic].

Sets the legend display style. This setting is effective when the numerical value display is selected in the [Legend Style] pull-down menu.

Fine-adjusts the positioning of the legend display from the top edge.

Sets the width of the lines displayed in the legend column.

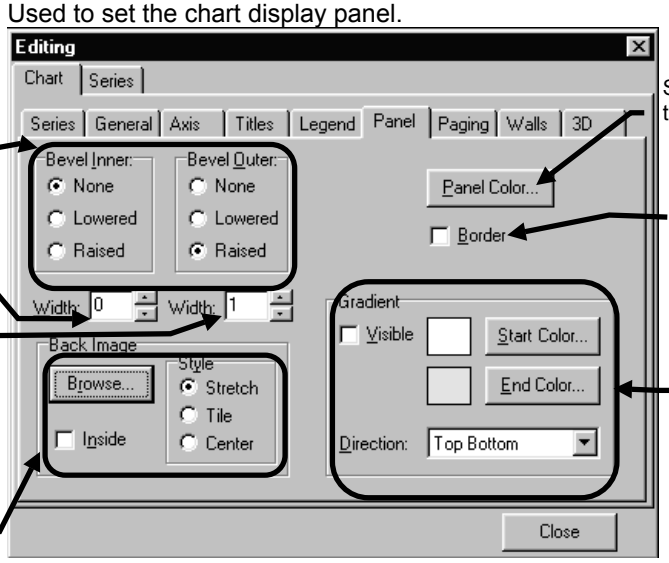
Sets the type, color and width of the lines dividing the legend items.

Enables or disables the inversion of the display order of legend items.

Sets the color and width of the shadow area of the shadowed display of legend area.

- [Panel] sub-panel

Used to set the chart display panel.



Set the format of the borders of the chart display area. [Bevel Inner] sets the inner borders of the chart display area and [Bevel Outer] sets the outer borders of the chart display area.

Sets the width between the inner and outer borders.

Sets the width of the inner and outer borders.

Specifies the background image of the chart display area, sets whether it is displayed inside the chart or in the entire chart display area, and sets its display position.

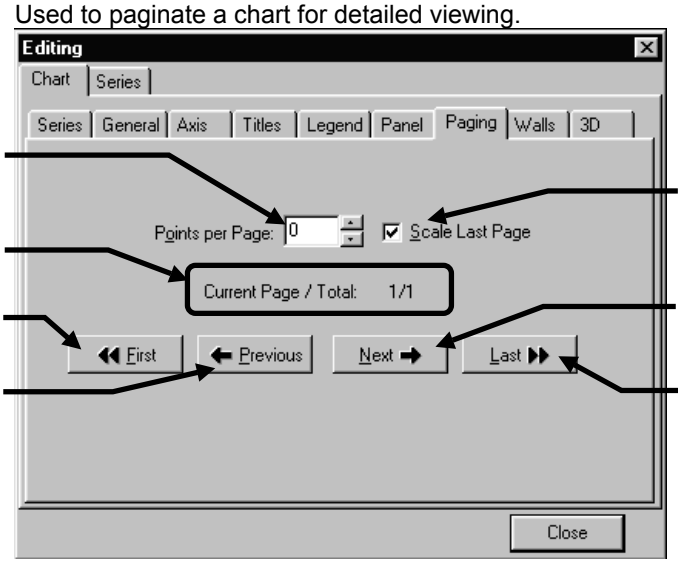
Sets the background color of the chart display area.

Enables or disables the display outside the outer frame of the chart display area.

Sets whether or not the chart display area background is displayed with gradations, and sets the colors and type of the background.

- [Paging] sub-panel

Used to paginate a chart for detailed viewing.



Sets the number of horizontal axis points displayed per page.

Sets the current page number and total number of pages.

Moves to the first page.

Moves to the previous page.

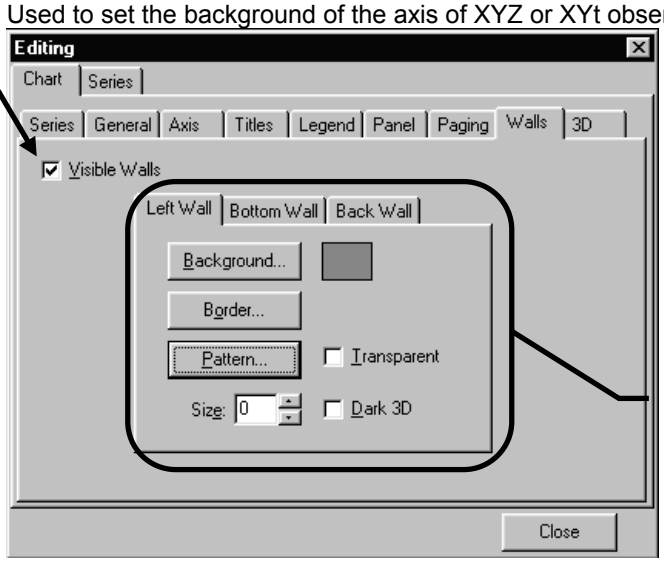
Enables or disables the change in scaling in the last page.

Moves to the next page.

Moves to the last page.

- [Walls] sub-panel

Used to set the background of the axis of XYZ or XYt observation chart.



Enables or disables the chart axis background display.

Changes color of Left, Bottom and Back wall.

<Background> button:
Sets the background color.

<Border> button:
Sets whether or not the background frame is displayed, and sets its color, width and type.

<Pattern> button:
Sets whether or not the background pattern is displayed, and sets it color, width and type.

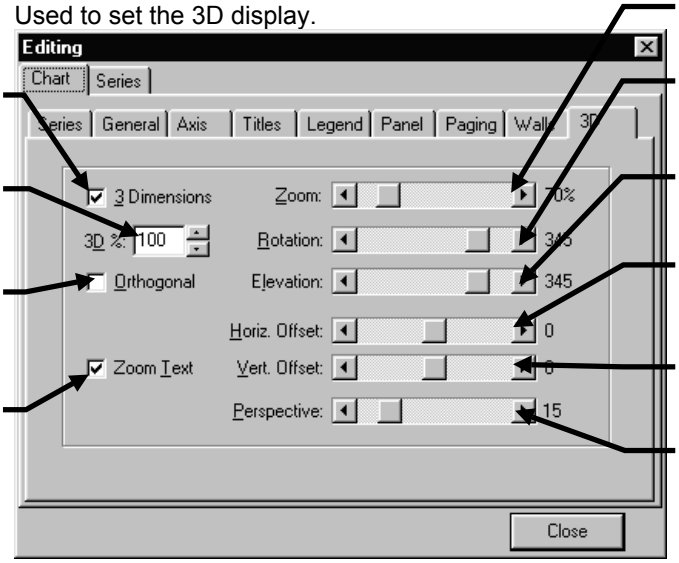
[Transparent] check box:
Enables or disables the transparent background display.

[Size] text box:
Sets the background thickness.

[Dark 3D] check box:
Enables or disables the shadowed, 3D display.
This check box can be selected when the background thickness is specified.

- [3D] sub-panel

Used to set the 3D display.



Switches between the 3D and 2D chart display.

Sets the 3D chart display effect by varying the angle.

Enables or disables the 3D display in the depth-wise direction.

Enables or disables the zooming display of character strings in the chart, such as label characters, together with the chart display.

Change the zoom ratio.

Rotates the chart in the horizontal direction with respect to the bottom side.

Rotates the chart in the vertical direction with respect to the bottom side.

Moves the chart display position in the horizontal direction.

Moves the chart display position in the vertical direction.

Distorts the chart to change the display effect.

2-15-2 [Series] Panel

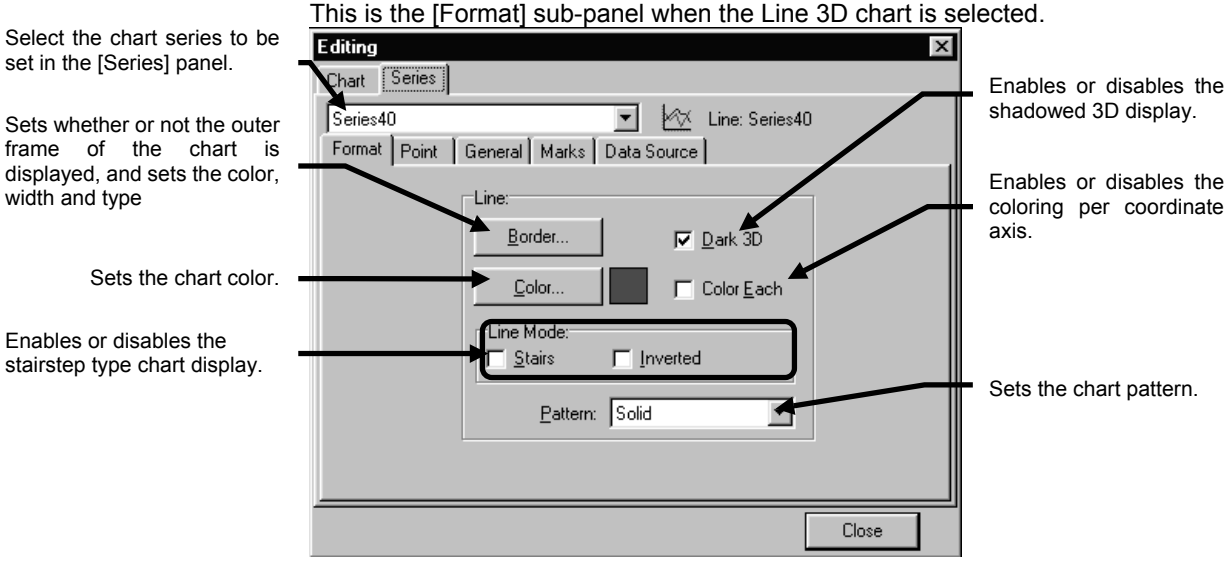
- [Format] sub-panel

  Used to set the display method of a series of images.

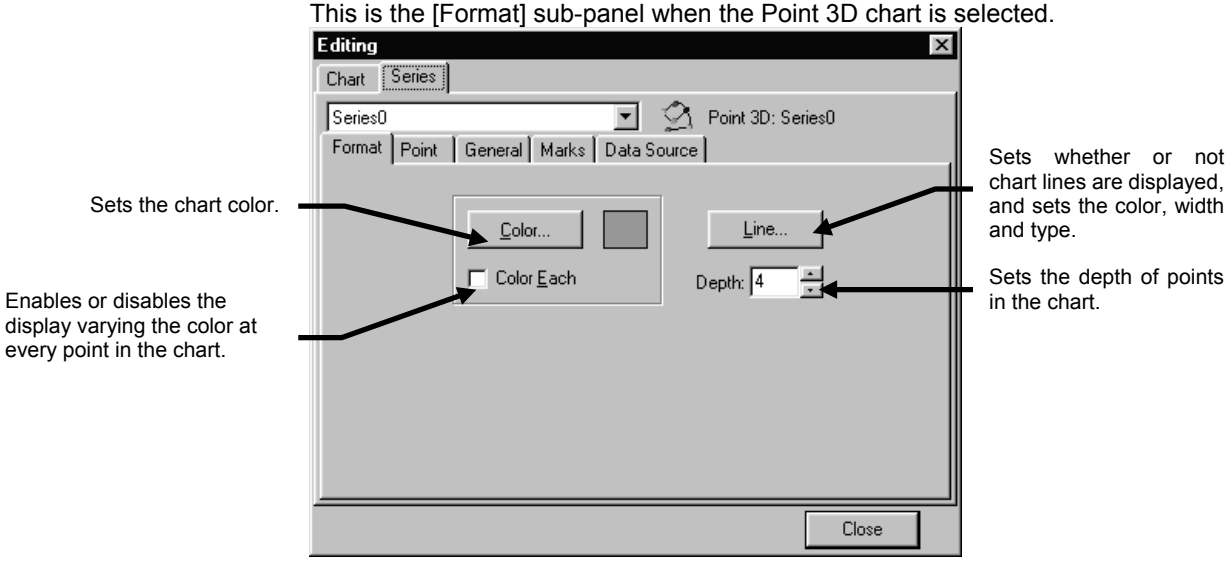
  The set items are variable depending on the type of the chart.

  The following descriptions take a Line 3D chart and Point 3D chart as examples.

This is the [Format] sub-panel when the Line 3D chart is selected.

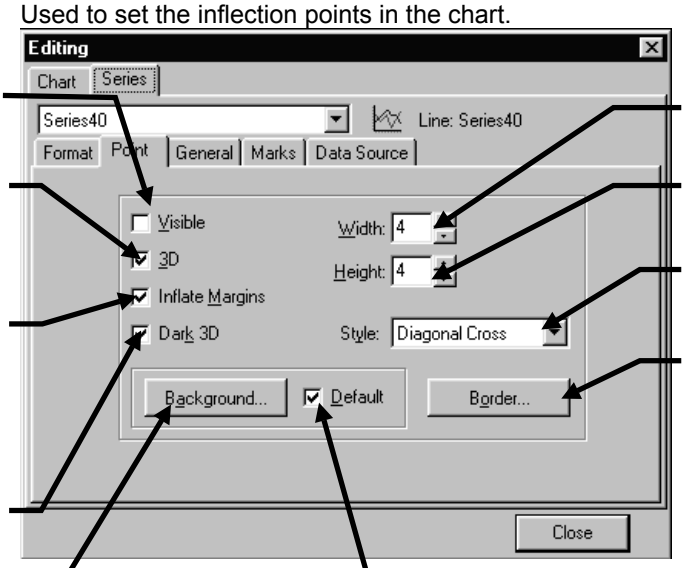


This is the [Format] sub-panel when the Point 3D chart is selected.



- [Point] sub-panel

Used to set the inflection points in the chart.



Enables or disables the inflection point display.

Enables or disables the 3D inflection point display.

Selects whether the ends of the inflection points are aligned with the coordinate axis (when checked) or the centers of the inflection points are aligned with the coordinate axis (when unchecked).

Enables or disables the dark shadowed 3D display of inflection points.

Sets the inflection point background color.

Resets the background color to the default color.

Sets the width of inflection points.

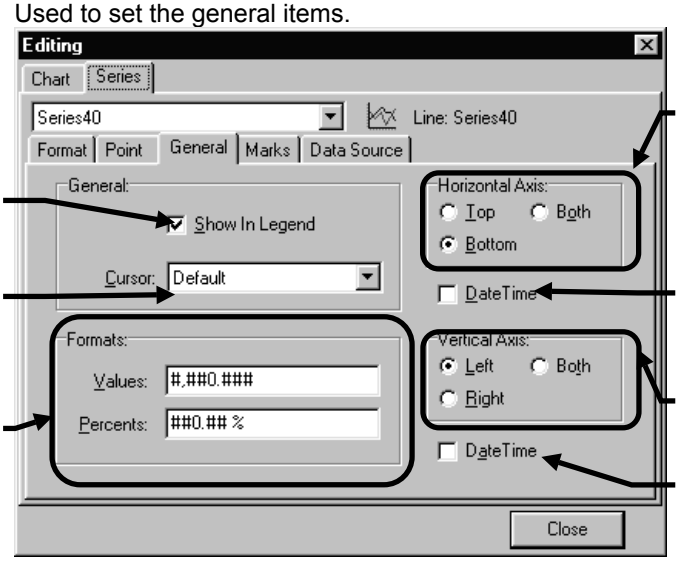
Sets the height of inflection points.

Sets the type of inflection points.

Sets whether or not the inflection point borders are displayed, and sets the color, width and type.

- [General] panel

Used to set the general items.



Enables or disables the chart display inside the legend.

Sets the shape that the cursor changes when it is placed on the chart.

Sets the display formats when the label display is set to the numerical value display or percent display.

Selects whether the labels are displayed on or below the chart.

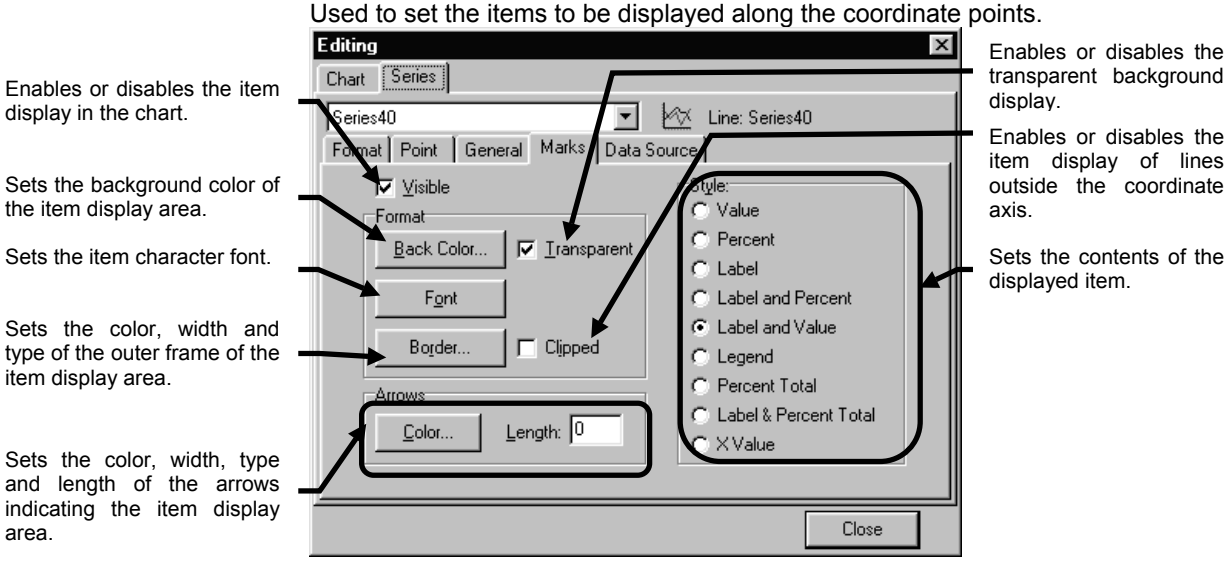
Usually leave this box unchecked.

Selects whether the labels are displayed to the left or right of the chart.

Usually leave this box unchecked.

- [Marks] sub-panel

  Used to set the items to be displayed along the coordinate points.



- [Data Source] sub-panel

  Do not change the settings here but leave them in the initial condition.

2-16 Pop-up Menus

Selection of function windows and image windows and other frequently-used FLUOVIEW functions (full-screen display, printer output, image save, LUT setting, comment setting) can be controlled by clicking the right button of the mouse, without selecting specific page tabs or buttons.

- Pop-up menu of comment

  When the right button of mouse is clicked on an comment in image, a pop-up menu appears to allow editing or deleting the specified comment.

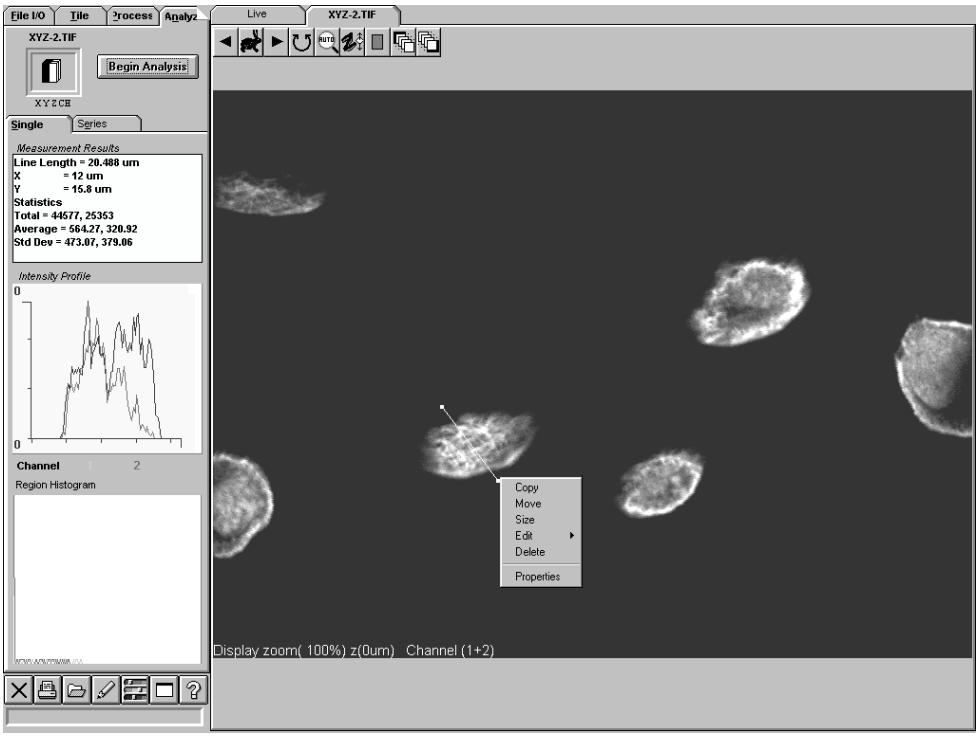
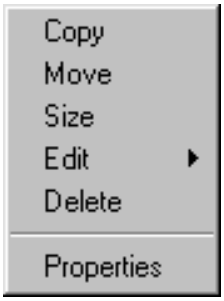


Fig. 2-152 Image Comment with Pop-up Menu



Copies the comment.

Moves the comment.

Resizes the comment.

Edits the selected arrow or scale.

Deletes the comment.

Edits the comment color and font.

- Pop-up menu of image

  When the right button of mouse is clicked on the image, a pop-up menu appears to allow selection of image operations (full-screen display, printer output, image save, LUT setting, number of image divisions, comment editing).

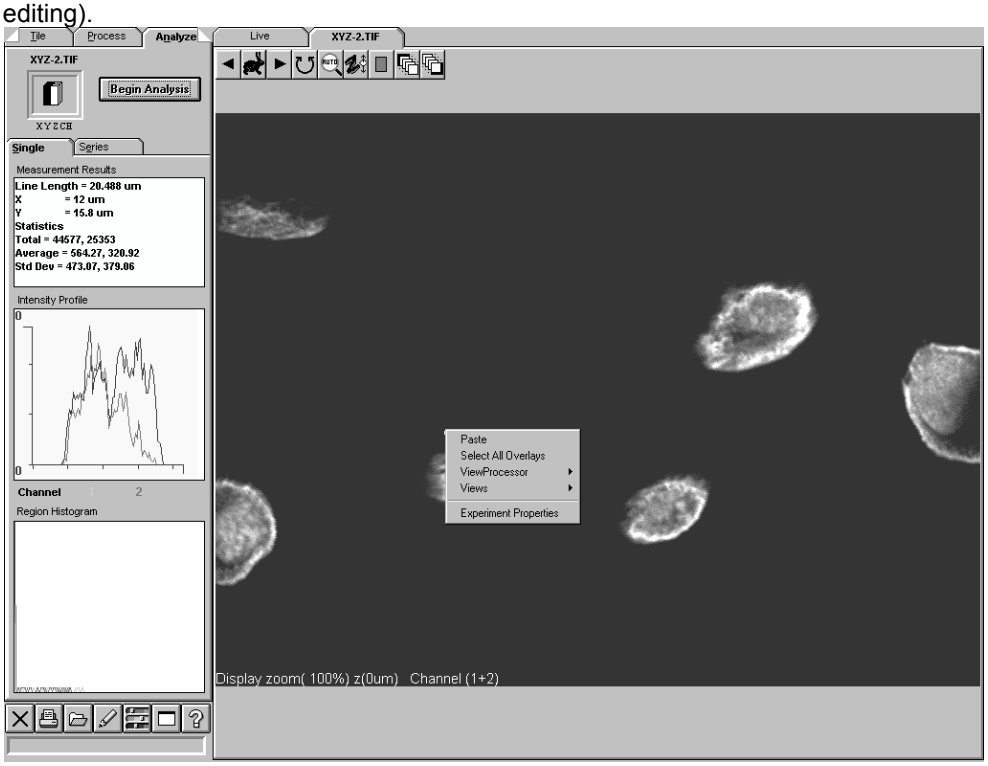


Fig. 2-153 Image Window Showing Pop-up Menu

Pasts the comment copied on the image.
Selects all the comments overlaid on the image.
Edits the image LUT.
Sets the number of image divisions when displaying more than one image.
Edits the image comment.

- Pop-up menu of the [Experiments in Memory] dialog box

  When the right button of mouse is clicked in the frame for opening an image file in the [File I/O], [Tile] or [Process] panel, a pop-up menu appears. This makes it possible to select the file to be opened when performing tile display, inter-image operations, image channel merger/extraction, etc.

Click the mouse right button here.

The pop-up menu appears showing the file names that can be selected.

Select the file name to be processed.

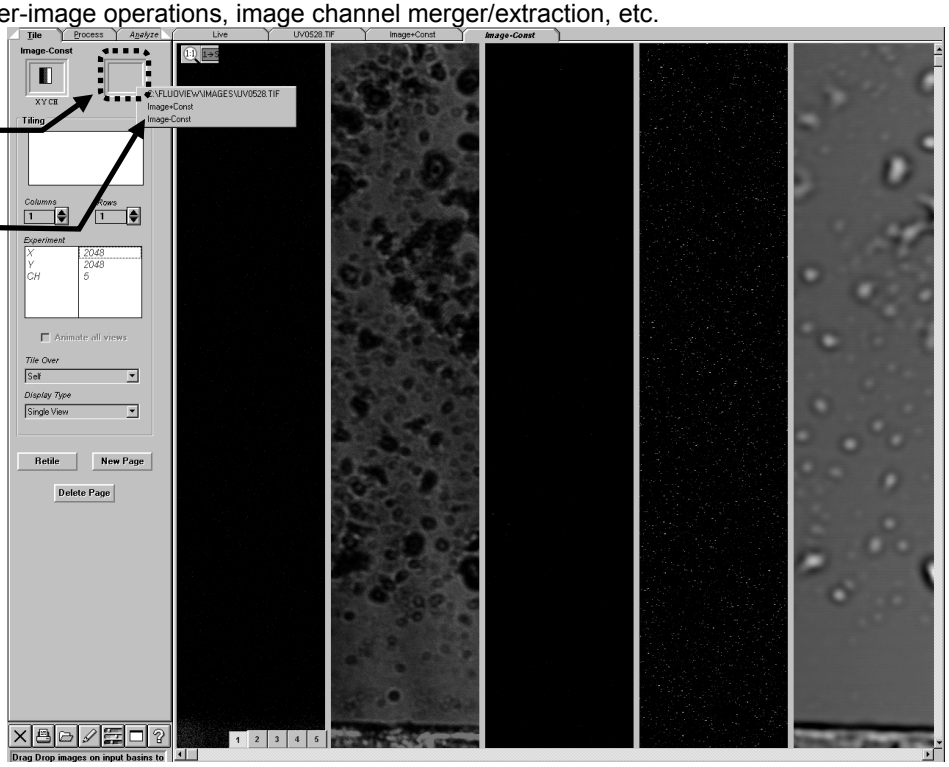


Fig. 2-154 Function Window Showing Pop-up Menu

Appendix A List of Hot Keys

Frequently-used FLUOVIEW functions (scanning, panel switching) can be controlled from the keyboard without using the mouse.

- Image acquisition-related keys

Image acquisition channel selection

Key	Target Operation
Ctrl + 1	Switches the status of the Ch1 check box (Ch1 scanned/not scanned).
Ctrl + 2	Switches the status of the Ch2 check box (Ch2 scanned/not scanned).
Ctrl + 3	Switches the status of the Ch3 check box (Ch3 scanned/not scanned).
Ctrl + 4	Switches the status of the Ch4 check box (Ch4 scanned/not scanned).
Ctrl + 5	Switches the status of the Ch5 check box (Ch5 scanned/not scanned).

PMT voltage/gain/offset adjustments

Key	Target Operation
Shift + ←	Enables and makes variable the LED slider on the left.
Shift + →	Enables and makes variable the LED slider on the right.
Alt + ↓	Decreases the value of an enabled LED slider. (Fine adjustment)
Shift + ↓	Decreases the value of an enabled LED slider. (Coarse adjustment)
Alt + ↑	Increases the value of an enabled LED slider. (Fine adjustment)
Shift + ↑	Increases the value of an enabled LED slider. (Coarse adjustment)

NOTE

An LED slider is enabled and variable when its setting values are displayed in red.

Image acquisition (scanning)

Key	Target Operation
F1	Displays the on-line help screen.
F2	Performs repeated scanning. [XY Repeat]
F3	Starts scanning according to the current scan mode. [Scan Once]
F4	Performs focus scanning. [Focus]
F6	Acquires another image after completing series image acquisition. [Append Next]
F7	Completes image acquisition after completing series image acquisition. [Series Done]
Space	Stops scanning. [Stop Scan]

Scanning speed and area setting

Key	Target Operation
Ctrl + →	Decreases the scan speed.
Ctrl + ←	Increases the scan speed.
Ctrl + ↓	Decreases the zoom ratio.
Ctrl + ↑	Increases the zoom ratio.

Z stage setting

Key	Target Operation
Page Up	Moves the Z stage up. (Fine adjustment)
Shift + Page Up	Moves the Z stage up. (Coarse adjustment)
Page Down	Moves the Z stage down. (Fine adjustment)
Shift + Page Down	Moves the Z stage down. (Coarse adjustment)
Ctrl + Page Up	Sets the Z stage to the Stop Z position.
Ctrl + Page Down	Sets the Z stage to the Start Z position.
Insert	Switches the [Locked] check box (motor excitation) status.
Ctrl + Delete	Cancels the Start and Stop settings. <Set Zero> Z motor: The current position becomes 0.0. Piezo: Returned to the 0.0 position.
Home	Moves the stage till the Stop Z position.
End	Moves the stage till the Start Z position.

- Transmitted illumination lamp ON/OFF keys

Key	Target Operation
Ctrl + L	Switches the transmitted illumination ON/OFF. <Trans. Lamp>

- Image save key

Key	Target Operation
Ctrl + S	Save the image in the image window being displayed as series images. <SAVE>

- [Hi-Lo]LUT switchover key

key	Target operation
Ctrl + H	It alternatively switches over between the previously-assigned LUT and [Hi-Lo] LUT on the channel, whose PMT adjustment window ([PMT], [Gain] and [Offset]) is opening on the [Acquire] panel during the acquisition period.
Ctrl + Shift + H	It alternatively switches over between the previously-assigned LUT and [Hi-Lo] LUT on all the channels acquiring images.

- Panel select keys

Main panel

Key	Target Operation
Alt + A	Selects the [Acquire] panel.
Alt + F	Selects the [File I/O] panel.
Alt + T	Selects the [Tile] panel.
Alt + P	Selects the [Process] panel.
Alt + N	Selects the [Analysis] panel.
Alt + V	Selects the [Visualize] panel.

[Acquire] sub-panels

Key	Target Operation
Alt + S	Selects the [Settings] sub-panel.
Alt + Z	Selects the [Z Stage] sub-panel.
Alt + M	Selects the [Time Series] sub-panel.
Alt + D	Selects the [Dyes] sub-panel.
Alt + L	Selects the [Lasers] sub-panel.
Alt + E	Selects the [TIEMPO] sub panel . (It is an option panel with TIEMPO available).
Alt + C	Selects the [Spectral Detector] panel.(When using a spectral detecting system)

[Process] sub-panels

Key	Target Operation
Alt + M	Selects the [Math] sub-panel.
Alt + I	Selects the [Filter] sub-panel.
Alt + H	Selects the [Histogram] sub-panel.
Alt + K	Selects the [Mask] sub panel. (It is an option panel with TIEMPO available).
Alt + X	Selects the [Experiment Editor] sub panel.

[Analysis] sub-panels

Key	Target Operation
Alt + S	Selects the [Single] sub-panel.
Alt + E	Selects the [Series] sub-panel.
Alt + I	Select the [Isoplot] sub panel. (It is an option panel with TIEMPO available).

[Visualize] sub-panels

Key	Target Operation
Alt + O	Selects the [Orientations] sub-panel.
Alt + R	Selects the [Other options] sub-panel.

Image window sub-panels

Key	Target Operation
Alt + G	Opens Gallery window.
Alt + U	Opens User Preferences.
Alt + → / ←	Changes image windows.
Ctrl + N	Creates a new image window whose content is same as that of the active image.

Appendix B Glossary

A

AOTF
AOTF represents Acoustic Optical Tunable Filter.
AOTF is the sound optical element having optical anisotropy. When a sound wave is spread in the element, the element behaves as a phase grid and only the light of the wavelength corresponding to the wavelength of a sound wave is diffracted in the specific direction. Therefore, it is possible to control selection of laser wavelength and adjustment of laser intensity at high speed.

Active
Status of being selected or executable. An active window can be distinguished from other windows by the color of the title bar.

Application
Same as “software”. Among software, refers to the software used directly by the users.

B

Backlash
The quantity of play (or loosening between gear teeth and part) which is produced when the stage is moved up or down by the Z-motor.

Button
→Command button.

C

Check box
A small square which can be either checked with ✕ or cleared. The check box indicate an item which can be enabled or disabled. The item is enabled when it is checked with X.

Clear
Action of removing check mark ✕ from a check box to disable the item. To clear a check box checked with ✕, click the check box.

Click
Action of pressing then releasing the button of the mouse.

Clipboard
The place which relays data when an operation such as “Copy”, “Cut” and “Paste” is executed.

Command button
A figure in the shape of button in the window. Clicking a command button with the mouse allows the function indicated on the button to be executed.

Confocal
Signifies the possibility of obtaining data on the plane where the irradiated laser beam is focused.

Contrast
Variation (change) between the brightest and darkest areas in an image.

Control menu
The menu displayed when the control menu box at the left end of the title bar of a window is clicked. When the window is minimized, the control menu can be displayed by clicking the icon. The control menu contains commands for controlling the window.

Control menu box
The square button at the left end of the title bar of a window. Clicking this box opens the control menu.

Copy
Action of placing selected data in the clipboard so that the data can be placed in other place later.

Cursor
Blinking | which indicates the area where a character can be input. When a keyboard key is pressed, the character is entered in the position of the cursor.

D

Dialog box
Some functions require fine settings so that they can be executed, and some functions require the confirmation of settings before being executed. The dialog box is a sub-window displayed in such cases.

Directory
Hierarchical classification of the space inside a disk so that the files can be arranged in a significant manner according to their categories.

Disk drive
The storage device storing the files. Some disk drives such as the hard disk drive and floppy disk drive are capable of both input and output, and some such as the CD-ROM drive is designed for read only.

Dot
→Pixel.

Double-click
Action of pressing and releasing the mouse button quickly twice, without moving the moue.

Drag
Action of placing the mouse pointer on the target function, pressing the mouse button, moving the mouse while keeping the mouse button pressed, and releasing the mouse button at the destination position.

Drive
→Disk drive.

Drive name
Character such as “A” and “C”, assigned to each drive.
→ Disk drive.

E

Extended focus
View of an XYZ image obtained by projection in the Z-direction.

Extension
Up to 3 characters after a period, which are attached at the end of a file name. The extension usually represents the type of the file or directory.

F

File
Group of information which is named and saved in a disk

G

Gain
This function brightens the image by the ratio set at the time of image acquisition. Use Gain when a bright image cannot be obtained even by setting PMT Voltage to 800 V.

Group
Refers to the applications registered in the program manager. When a group is opened on the program manager, more than one icon is displayed in the window. When the group is closed, the group becomes an icon of the window.

I

Icon
An icon is a small figure with characters below it. The icon indicates the status in which the window is closed (or minimized).

Iconize
This refers to turn a window into an icon display by using the iconize button of the [Iconize] command in the control menu. The application continues to run even after it has been turned into an icon. Selecting an icon returns it to the active application.

Intensity
Brightness of each pixel in an image.

Items displayed in pale color
The menu commands and buttons which cannot be used are displayed in less visible way, i.e. in a pale color or gray.

K

Keyboard input
Action of inputting an alphanumeric character from the keyboard of the computer.

L

LUT(LookUpTable)
The image acquired by observation (input) has 12 bits of brightness data per pixel. Meanwhile, the brightness data which can actually be displayed (output) consists of 8 bits per pixel for each of R, G and B. The LUT is the tabulation of this relationship between the input and output.

M

Macro
Record of a series of operations. When a macro is executed, the series of operations defined for it are executed.

Maximize button
The button showing an upward triangle at the right of the title bar. Clicking this button displays the window full screen. The same operation is also available using the [Maximize] command in the control menu.

Mouse
A device which was named because it looks like a mouse. It is used to give instructions in the window.

Mouse pointer
When the mouse is moved on the desk, arrow ↖ moves in the display along the movement of the mouse. This "↖" is called the mouse pointer.

O

Offset
This function darkens the image by the ratio set at the time of image acquisition.
(Offset should be used before using Gain.)

On-line help
Manual displayed on the screen, that is built into software.

Option button
Small circular button inside a rectangular frame in a window. Only one option button (item) can be selected from the items enclosed in the frame.

P

Panel
The large rectangle with a page tab in a window. The panel is provided on a per-function basis, and clicking the page tab displays the panel of the selected function.

Paste
Action of placing the data in the clipboard in an application.

Piezo-
Abbreviation of piezoelectric, which is the phenomenon of generating electricity when a force is applied. This phenomenon is utilized in electric spark generators for use with gas appliances, etc.

Pixel
The minimum graphic unit of screen display. Also referred to as the dot.

PMT Voltage
Increasing this setting increases the sensitivity.
When a bright image cannot be obtained even by setting PMT Voltage to 800 V, do not vary PMT Voltage any more but increase Gain. This usually provides a better effect than increasing PMT Voltage above 800 V.

R

Resolution
Number of dots composing the image on a screen or printer. When the number of dots is increased, i.e. when the resolution is increased, the gradation can be displayed in more details.

Reversed display
Display method which indicates that an item or character string is selected and has become the target of the user's next operation. The reversed displayed characters are shown in a different color from other characters.

S

Scroll
Action of moving text or a picture up or down in order to view the other part of information than the information which can be displayed at once in the window.

Scroll bar
The bar displayed at the bottom or right of a window containing more information than can be displayed at once. The scroll bar has a knob and two arrow buttons. Dragging the knob scrolls the information directly and clicking one of the arrow buttons scrolls the information line by line. Holding an arrow button scrolls the information continuously.

Simulated colors
Colors used to display the image data acquired by observation on a display. Original simulated colors can be created by editing the LUT.

Status bar
The line showing information at the bottom of a window. It shows the information on the operation or the description of the function selected with the mouse pointer.

T

Text
A file expressed with the ASCII codes such as characters and numerals, and with some control codes such as the line feed code. This format is referred to as the text format. The text is usually input from the keyboard.

Text box
A box in the window that accepts the input of character strings. Clicking a text box displays blinking |. This "|" indicates the position where the input character is inserted.

Title bar
The horizontal bar at the top of the window, that shows the title of the window or dialog box.

Toolbar
The toolbar provides frequently used functions in the form of buttons. It can be used any time during execution of any function.

W

Window
A large rectangle with a title. A window can be opened or closed.

Appendix C USER REGISTRATION OF FV1000

FLUOVIEW FV1000 can store the system setup information (PMT Voltage, Gain, Offset, etc.) on a per-user basis. To make this possible, you have to register yourself as a user and log in personally when starting up the FV1000 software.

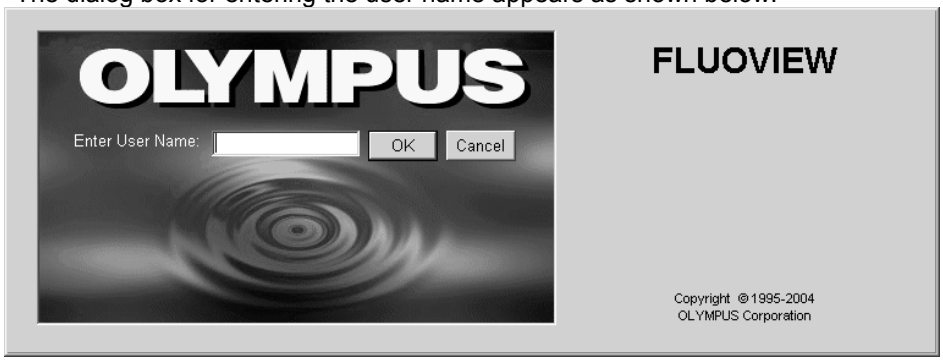
Appendix C-1 User Registration

Register yourself as a new user.

[FLUOVIEW Setup] icon

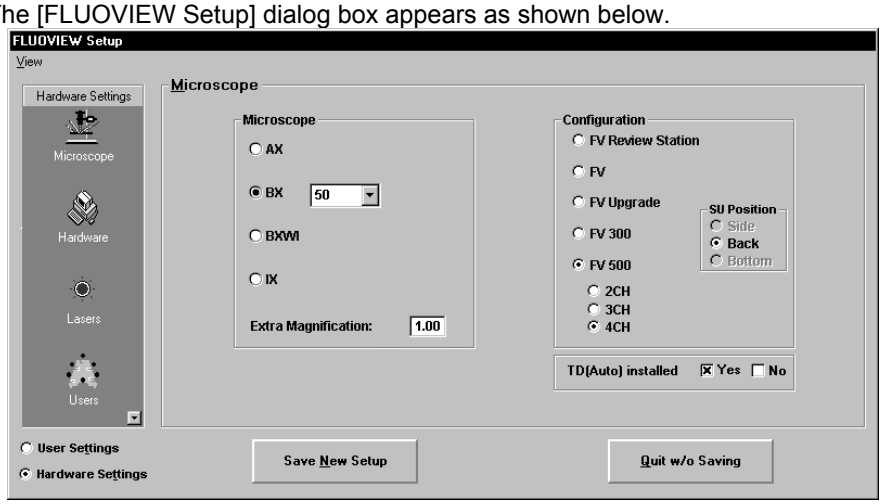
1. Double-click the [FLUOVIEW Setup] icon on the desktop.

   The dialog box for entering the user name appears as shown below.

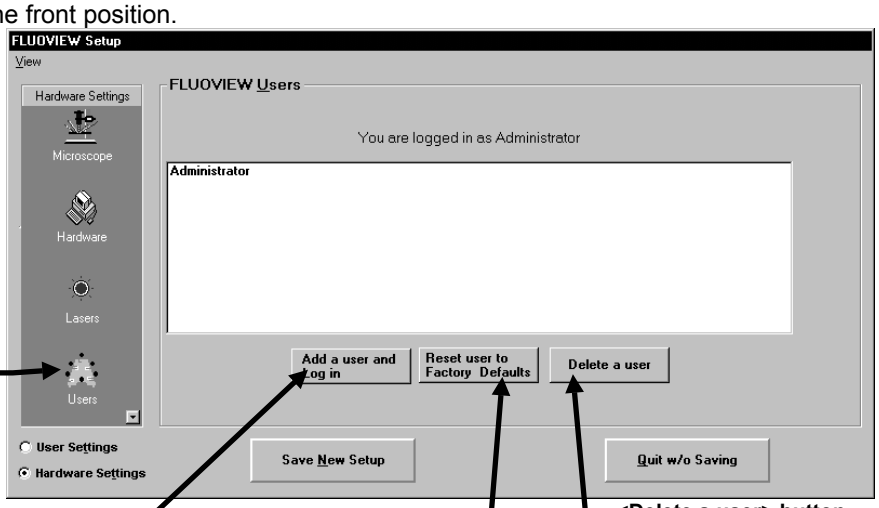


2. Enter “Administrator” in the [User Name:] text box and click the <OK> button.

   The [FLUOVIEW Setup] dialog box appears as shown below.



3. Click the [Users] icon in [Hardware Settings] to display the [FLUOVIEW Users] panel in the front position.



[Users] icon

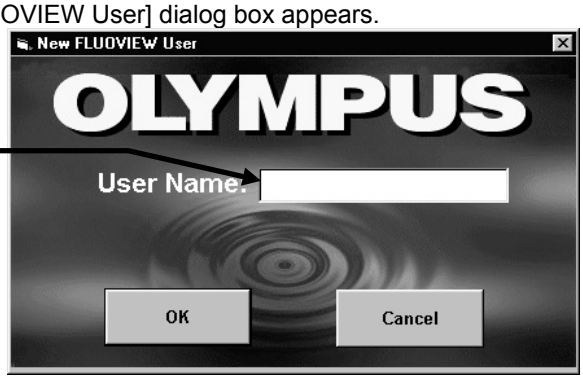
<Add a user and Log in> button
Used to add a new user.

<Reset user to Factory Defaults> button
Resets the system setups of the users selected in the list box to the factory defaults.

<Delete a user> button
Deletes the user selected in the list box. (The Administrator and the user who log in now cannot be deleted.)

4. Click the <Add a user and Log in> button.

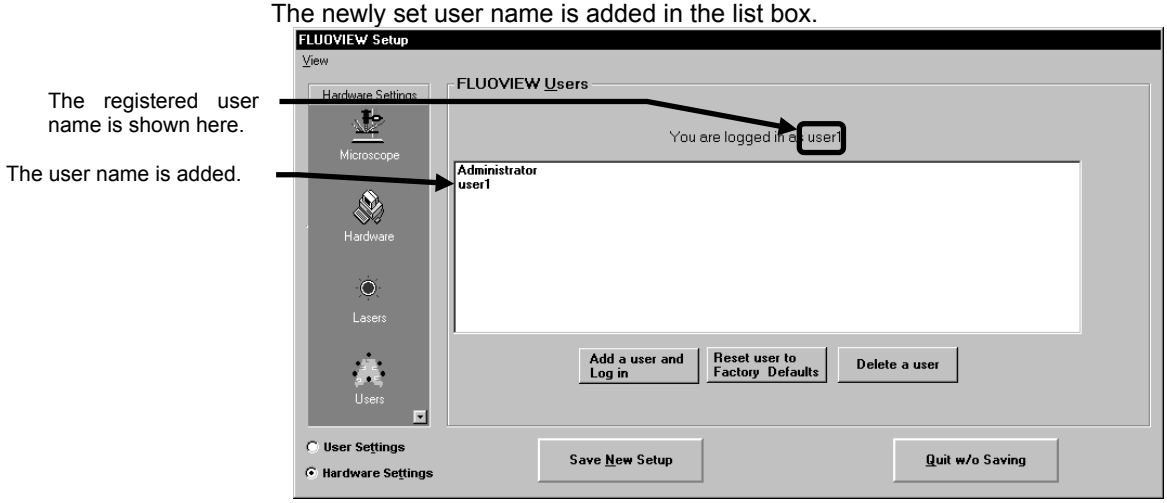
5. The [New FLUOVIEW User] dialog box appears.



[User Name:] text box
Enter the user name to be registered.

6. Enter the user name in the [User Name] text box and click the <OK> button.

The newly set user name is added in the list box.



7. To register other users, repeat steps 3 to 5 for each one.

8. Click the <Save New Setup> button or <Quit w/o Saving> button to close the dialog box.

TIP The Administrator is the user name which saves the factory defaults of the system setup.

Appendix C-2 Logging into the FV1000

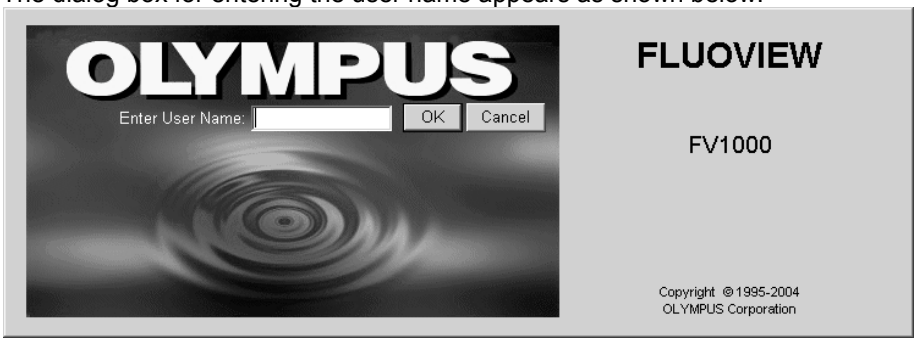
After the user name has been registered and the FV1000 started, a dialog box for entering the user name appears.

Enter the user name to log in the FV1000.

[FLUOVIEW] icon

1. Double-click the [FLUOVIEW] icon on the desktop.

   The dialog box for entering the user name appears as shown below.



2. Enter the user name in the [User Name:] text box and click the <OK> button.

3. The FLUOVIEW software starts.

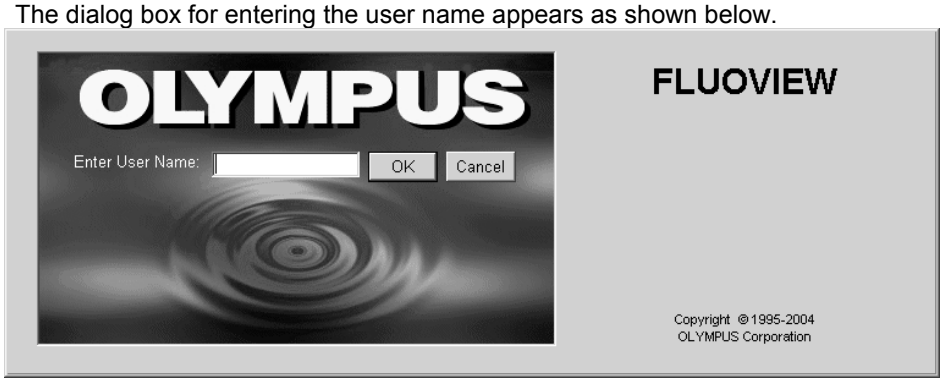
TIP If no user name has been registered, the [FLUOVIEW User Login] dialog box is not displayed, but the system is automatically started for the Administrator.

Appendix C-3 Deleting a User

[FLUOVIEW Setup] icon

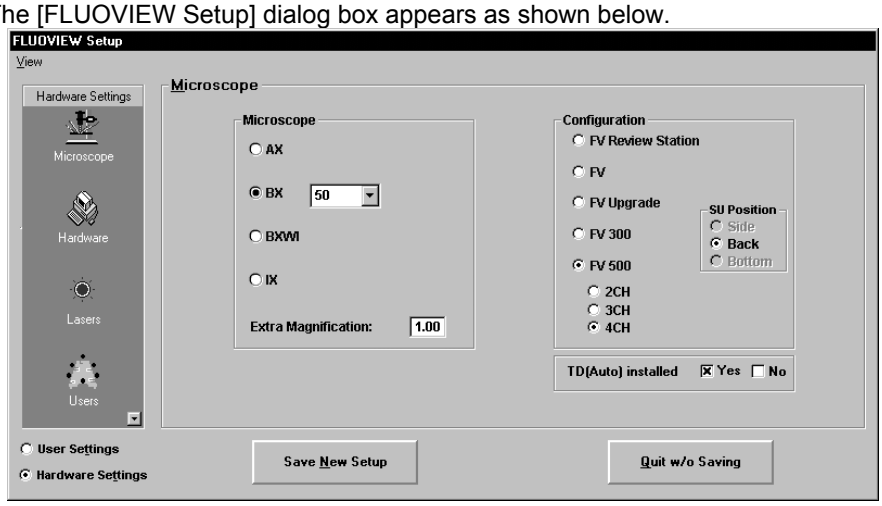
1. Double-click the [FLUOVIEW] icon on the desktop.

   The dialog box for entering the user name appears as shown below.

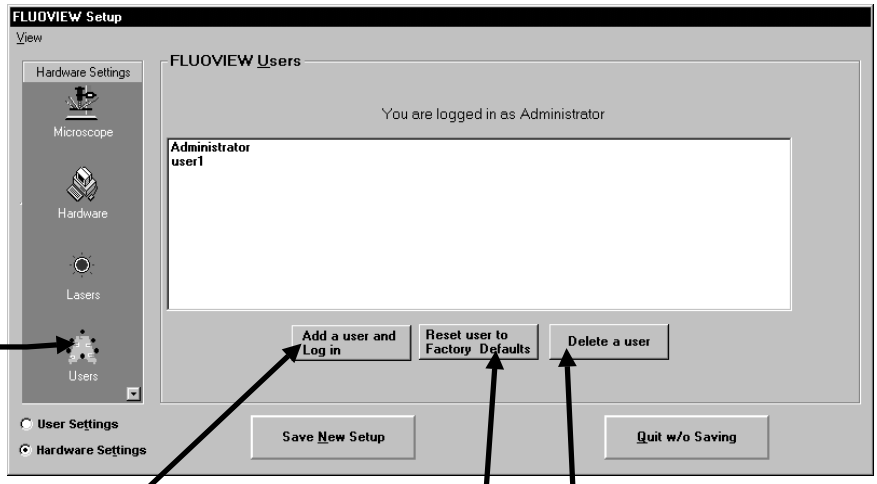


2. Enter “Administrator” in the [User Name:] text box and click the <OK> button.

   The [FLUOVIEW Setup] dialog box appears as shown below.



3. Click the [Users] icon in [Hardware Settings] to display the [FLUOVIEW Users] panel at the front.



[Users] icon

<Add a user and Log in> button
Used to add a new user.

<Reset user to Factory Defaults> button
Resets the system setups of the users selected in the list box to the factory defaults.

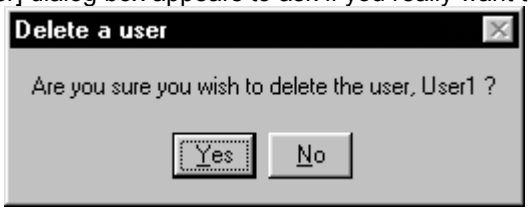
<Delete a user> button
Deletes the user selected in the list box.
(The Administrator and the user who log in now cannot be deleted.)

4. Select the user name to be deleted from the list box.

5. Click the <Delete a user> button.

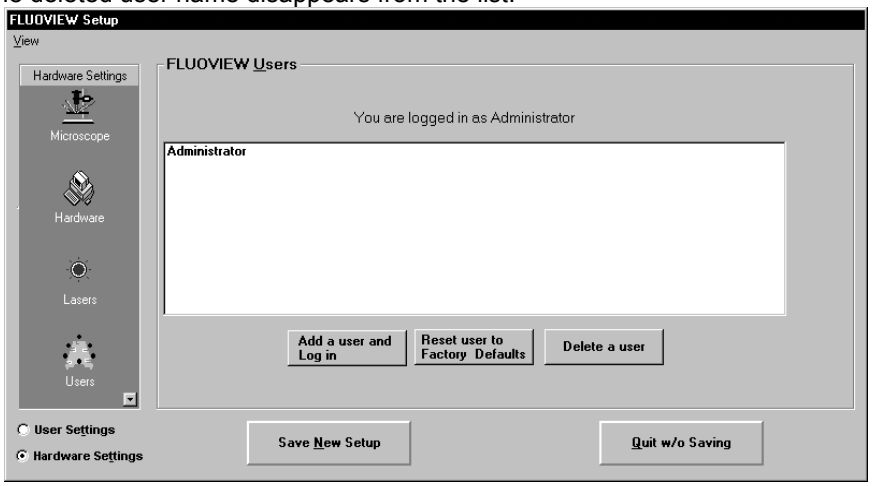
NOTE **The Administrator cannot be deleted from the list.**

6. The [Delete a user] dialog box appears to ask if you really want to delete the user.



7. Click the <Yes> button if you want to delete the user or the <No> button if you do not.

   The deleted user name disappears from the list.



NOTE **The user is deleted at the moment the <OK> button is clicked.**

8. Click the <Save New Setup> button or <Quit w/o Saving> button to close the dialog box.

Appendix D Change of Default Folder for [File I/O] Panel

FLUOVIEW FV1000 usually opens the default folder for the [File I/O] panel (C:\ FLUOVIEW \ IMAGES) to save acquired images or load saved images.

The default folder for saving an image can be changed or a desired folder can be specified directly when loading a saved image.

TIP

Change the default folder only when required.

There is no need of change if folder C: \ FLUOVIEW \ IMAGES is all right.

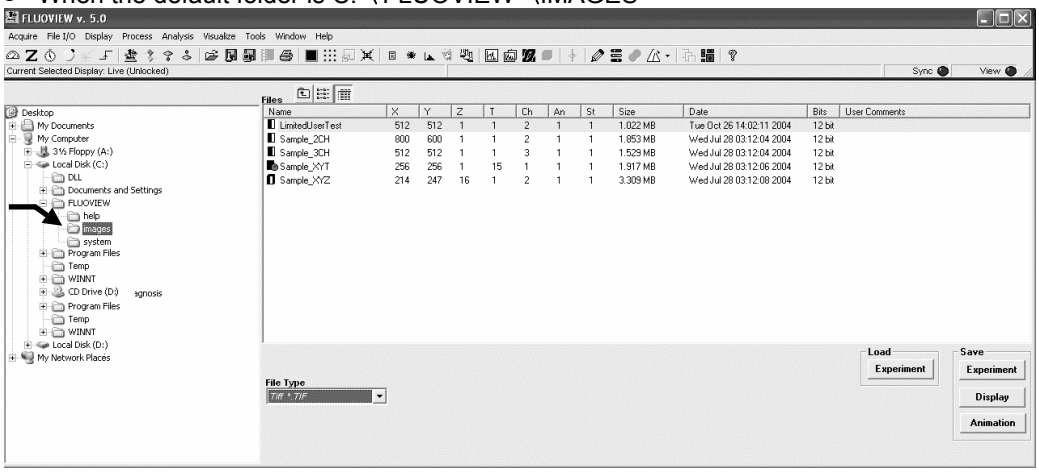
TIP

Folder names are delimited with " \".

For example, "C:\ FLUOVIEW \ IMAGES" means folder "IMAGE" in folder "FLUOVIEW" in "drive C".

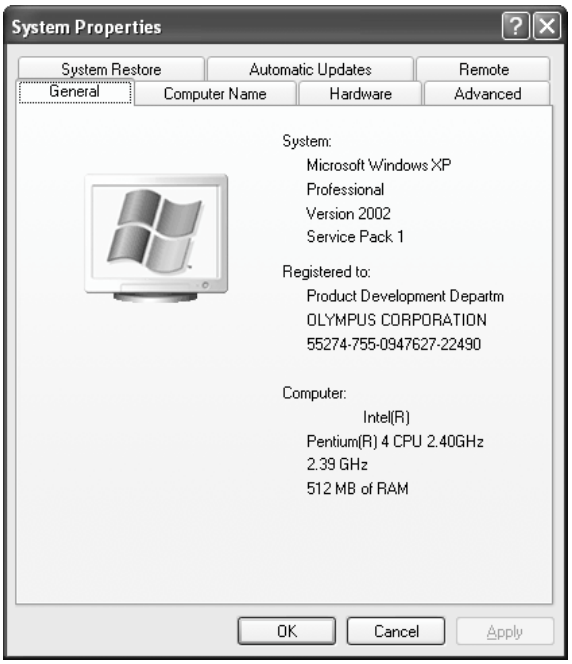
- When the default folder is C: \ FLUOVIEW \IMAGES

Default folder "C: \ FLUOVIEW \ IMAGES" is being open.

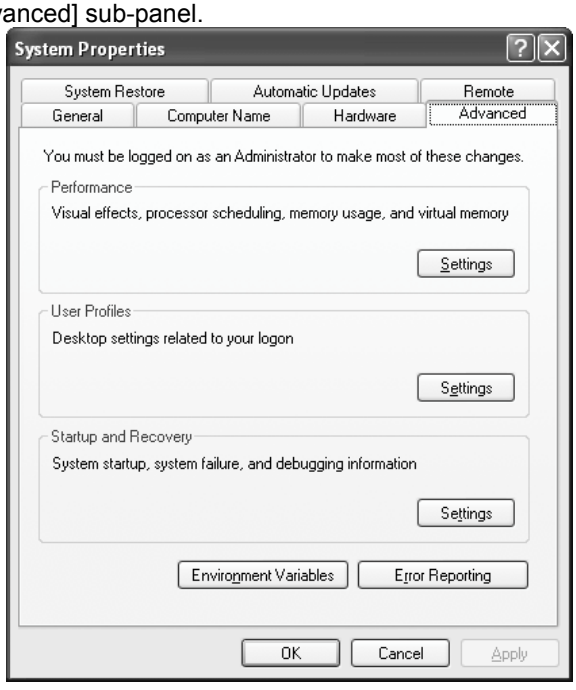

FLUOVIEW v. 5.0
Acquire File I/O Display Process Analysis Visualize Tools Window Help
Current Selected Display: Live (Unlocked)
Sync
View
Files
Desktop
My Documents
My Computer
3½ Floppy (A:)
Local Disk (C:)
DLL
Documents and Settings
FLUOVIEW
help
images
system
Program Files
Temp
WINNT
CD Drive (D:)
Program Files
Temp
WINNT
Local Disk (D:)
My Network Places
Name X Y Z T Ch An St Size Date Bits User Comments
LimitedUserTest 512 512 1 1 2 1 1 1.022 MB Tue Oct 26 14:02:11 2004 12 bit
Sample_2CH 800 600 1 1 2 1 1 1.853 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_3CH 512 512 1 1 3 1 1 1.529 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_XYT 256 256 1 15 1 1 1 1.917 MB Wed Jul 28 03:12:06 2004 12 bit
Sample_XYZ 214 247 16 1 2 1 1 3.309 MB Wed Jul 28 03:12:08 2004 12 bit
Load
Experiment
Save
Experiment
Display
Animation
File Type
TIF * TIF

A different default folder can be set for each of the users logging in Windows .

1. Click the [Start] button to open the [Start] menu. Then select commands [Settings] - [Control Panel] - [System]. The [System Properties] dialog box appears as shown below.



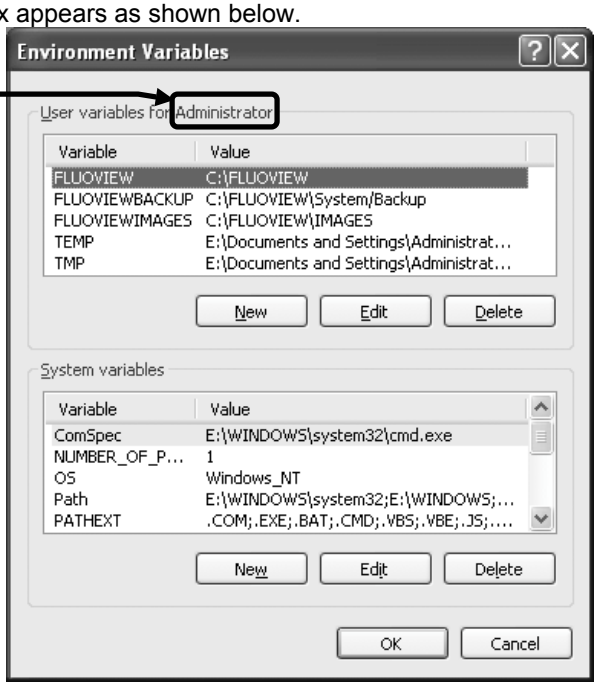
2. Select the [Advanced] sub-panel.



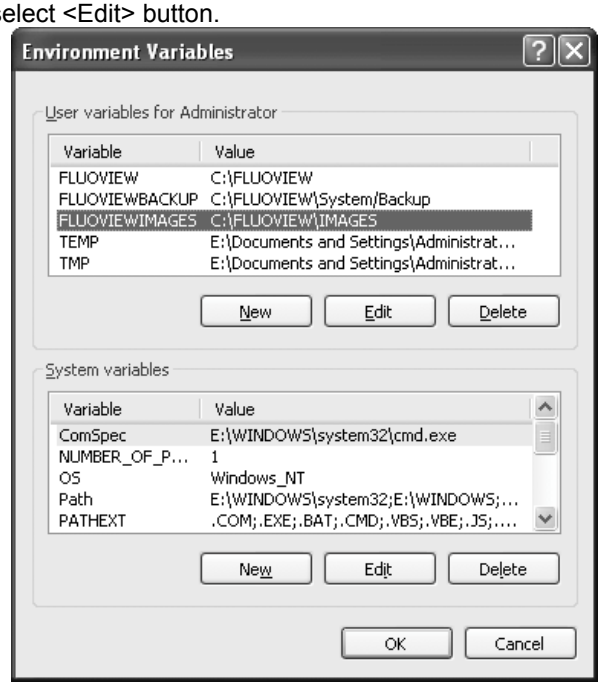
3. Select the <Environment Variables> button.

   The dialog box appears as shown below.

User name logging in Windows XP or Windows 2000.



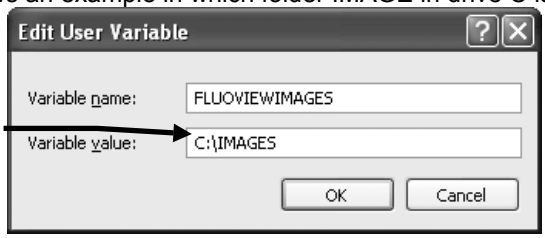
4. Select [FLUOVIEWIMAGES] under [Variable] in the [User variables for Administrator:] list box, and select <Edit> button.



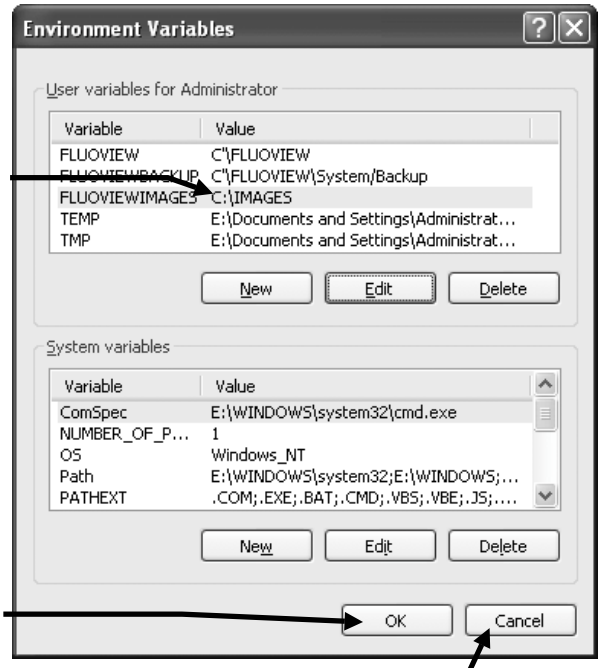
TIP "Administrator" of [User Variables for Administrator:] is variable depending on the user name logging in Windows XP or Windows 2000.

5. In the [Variable value:] text box, enter the path name of the default folder to be changed by delimiting the drive and folder names using backslashes (\ ).
(The figure shows an example in which folder IMAGE in drive C is entered.)

[Variable value:] text box
Enter the path name of the default folder.



6. Click the <OK> button



Change is reflected.

<OK> button

Saves the change and closes the dialog box.

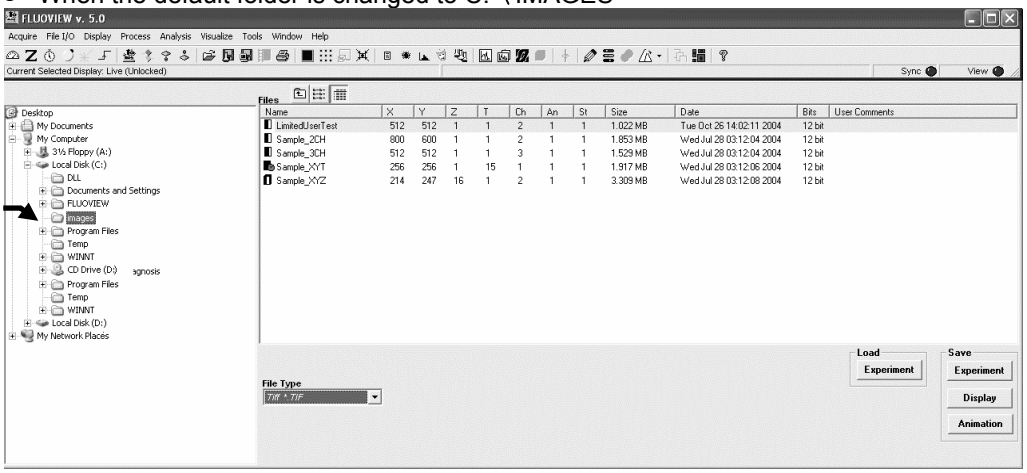
<Cancel> button

Closes the dialog box without saving the change.

7. Click the <OK> button to close the [Environment Variables] dialog box.
   Now, the default folder for the [File I/O] panel will be changed from the next time you start FLUOVIEW.

- When the default folder is changed to C: \ IMAGES

New default folder C \IMAGES is being open.


FLUOVIEW v. 5.0
Acquire File I/O Display Process Analysis Visualize Tools Window Help
Current Selected Display: Live (Unlocked)
Sync
View
Files
Desktop
My Documents
My Computer
3½ Floppy (A:)
Local Disk (C:)
DLL
Documents and Settings
FLUOVIEW
images
Program Files
Temp
WINNT
CD Drive (D:)
Program Files
Temp
WINNT
Local Disk (D:)
My Network Places
Name X Y Z T Ch An St Size Date Bits User Comments
LimitedUserTest 512 512 1 1 2 1 1 1.022 MB Tue Oct 26 14:02:11 2004 12 bit
Sample_2CH 800 600 1 1 2 1 1 1.853 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_3CH 512 512 1 1 3 1 1 1.529 MB Wed Jul 28 03:12:04 2004 12 bit
Sample_XYT 256 256 1 15 1 1 1 1.917 MB Wed Jul 28 03:12:06 2004 12 bit
Sample_XYZ 214 247 16 1 2 1 1 3.309 MB Wed Jul 28 03:12:08 2004 12 bit
File Type
Load
Experiment
Save
Experiment
Display
Animation

TIP To reset the default folder to the factory setup, enter "C: \ FLUOVIEW \ IMAGES" in the [Value] text box.

NOTE **Do not change the setup after "FLUOVIEWIMAGES" in the [User Variables for <username>] list box. If erroneous setting is made here, the system will be unable to be started up.**

Appendix E List of Functions in the [Active Overlays] Dialog Box

Active Overlays is a kind of overlay function displayed on an image.
Active Overlays does not simply show the entered characters, but searches the image data related to the keyword specified in < > and displays the data values on the image.

The following setups are required to enable the use of Active Overlays.

Appendix E-1 Coordinate Position Data

Appendix E-1-1 X-Coordinate

The X-coordinate position with respect to the top left corner of the screen is displayed.

«Syntax»

<x[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “x” as the first character inside < >.
2. Add the following characters to set the display method.
   - hotspot : Display measurement points with “+” markings.
   - raw : Display data in pixel values.
   - calibrated : Display data in numerical values (µm).
   - units : Display the unit (pixels/µm).

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<x raw value>	**
<x hotspot raw value>	+**
<x hotspot calibrated value units>	+**µm

TIP The detailed display procedure is described in section 2-11-3, “Viewing the X- or Y-Coordinate Position of Image” in Volume [OPERATION].

Appendix E-1-2 Y-Coordinate

The Y-coordinate position with respect to the top left corner of the screen is displayed.

«Syntax»

<y[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “y” as the first character inside < >.
2. Add the following characters to set the display method.

   hotspot : Display measurement points with “+” markings.

   raw : Display data in pixel values.

   calibrated : Display data in numerical values (μm).

   units : Display the unit (pixels/μm).

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<y raw value>	…… **
<y hotspot raw value>	……..+**
<y hotspot calibrated value units>	……..+**μm
<x hotspot raw value>,<y raw value>	……+(**,**)

TIP

The detailed display procedure is described in section 2-11-3, “Viewing the X- or Y-Coordinate Position of Image” in Volume [OPERATION].

Appendix E-1-3 Other

Positions can also be displayed by entering the following keywords in the syntax in place of the X- and Y-coordinate positions described in sections J-1-1 and J-1-2.

1 Z Position

With cross-section related images such as XYZ images, the Z position with respect to the first image can be displayed.

«Syntax»

<z[[raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

2 T Position

With time-related images such as XYZ images, the T position with respect to the first image can be displayed.

«Syntax»

<t[[raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

3 Animation

With animation images created in the [Visualize] panel, the position with respect to the first image can be displayed.

«Syntax»

<Animation[[raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

Appendix E-2 Intensity Data

The intensity value can be displayed.

When images are overlapped, the intensity value of each image is accompanied with the channel number placed after it.

«Syntax»

<intensity[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “intensity” as the first character inside < >.
2. Add the following characters to set the display method.

   hotspot : Display measurement points with “+” markings.

   raw : Display data in values between 0 and 4095.

   calibrated : Display data in numerical values (µm).

   units : Display the unit if this has been set.

   (Same as the concentration computation in FV-TIEMPO)

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<intensity raw value>	..**
<intensity hotspot raw value>	..***(1),**(2)
	(When images are overlapped)

TIP

The detailed display procedure is described in section 2-11-2, “Viewing the Intensity Value of Image” in Volume [OPERATION].

Appendix E-3 Other

The following image data can be displayed in addition to the X- and Y-coordinate positions and intensity value.

Appendix E-3-1 Channel Number

The channel number can be displayed.

When images are overlapped, the displayed channel numbers are connected by the "+" markings.

«Syntax»

<Channel>

Appendix E-3-2 Objective Power

The objective power used in image capturing can be displayed.

«Syntax»

<Objective>

Appendix E-3-3 Date of Image Capturing

The date at which the image was captured can be displayed.

«Syntax»

<Date>

Appendix E-3-4 Time of Image Capturing

The time of the day at which image was captured can be displayed.

«Syntax»

<Time>

Appendix E-3-5 Image File Name

The image file name can be displayed.

«Syntax»

<Name>

Appendix F Hand Switch and Microscope Frame Function Allocation

When the U-HSTR2 hand switch, the BXBX61/IX81 microscope frames, and U-FH focus adjustment knob are shipped from the factory, the following functions have been allocated to their control buttons.

NOTE **These function allocations are valid only when the FLUOVIEW software is started.**

Appendix F-1 Hand Switch Functions

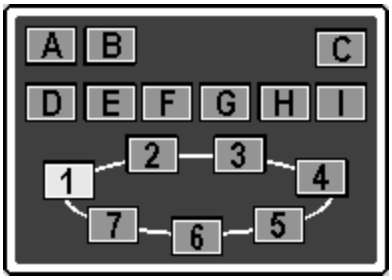


Fig. Appendix F-1 Hand Switch

Appendix F-1-1 BX/BXWI

Button	Function
A	Reflected light shutter OPEN/CLOSE switching
B	Top lens IN/OUT switching
C	Stage escape/return
D	Decreases the value of AS
E	Increases the value of AS
F	Condenser position down
G	Condenser position up
H	Cube position down
I	Cube position up
1 – 6	Objective setting
7	Not used

Appendix F-1-2 IX

Button	Function
A	Reflected light shutter OPEN/CLOSE switching
B	Not used
C	Not used
D	Filter wheel position down
E	Filter wheel position up
F	Condenser position down
G	Condenser position up
H	Cube position down
I	Cube position up
1 – 6	Objective setting
7	Not used

Appendix F-2 Microscope Frame Functions

Appendix F-2-1 BX

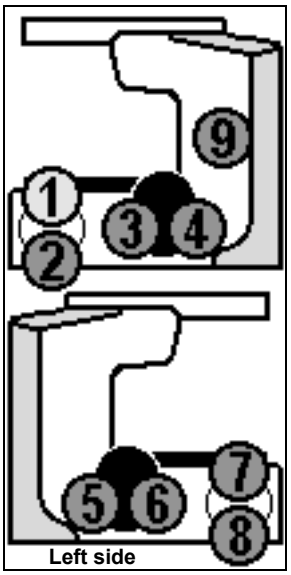


Fig. Appendix F-2 BX Microscope Frame

Button	Function
1	Transmitted light voltage up
2	Transmitted light voltage down
3	Z stage moves far from an objective lens.
4	Z stage nears to an objective lens.
5	Stage escape/return switching
6	Focus fine/coarse adjustment switching
7	Sets Light Preset position or normal position
8	Transmitted light ON/OFF switching
9	Not used

Buttons 3 and 4 are located on the right side of the microscope frame.

Appendix F-2-2 IX

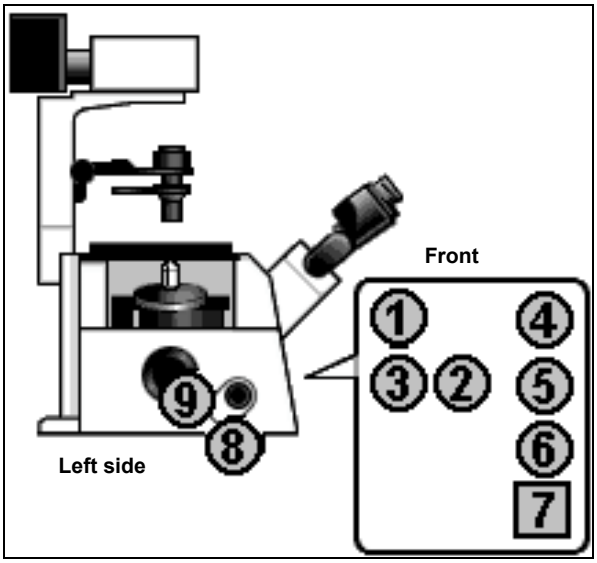


Fig. Appendix F-3 IX81 Microscope Frame

Button	Function
1	BI/Side or Back port light path switching
2	Objective lens nears to Z stage.
3	Objective lens moves far from Z stage.
4	Not used
5	Transmitted light voltage up
6	Transmitted light voltage down
7	Transmitted light ON/OFF switching
8	Focus fine/coarse adjustment switching
9	Objective lens escape/return switching

Appendix F-2-3 Focus Adjustment Knob

1 BX

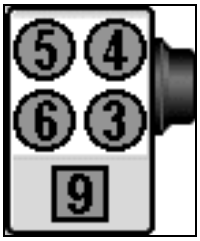


Fig. Appendix F-4 Focus Adjustment knob(BX)

Button	Function
3	Z stage moves far from an objective lens.
4	Z stage nears to an objective lens.
5	Stage escape/return switching
6	Focus fine/coarse adjustment switching
9	Not used

2 IX

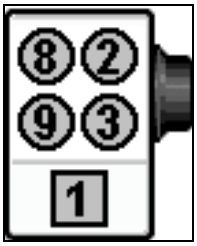


Fig. Appendix F-5 Focus Adjustment knob (IX)

Button	Function
1	BI/Side or Back port light path switching
2	Objective lens nears to Z stage.
3	Objective lens moves far from Z stage.
8	Focus fine/coarse adjustment switching
9	Objective lens escape/return switching

MAINTENANCE

On This Volume

This volume describes the user maintenance procedures of the FLUOVIEW FV1000 system.
Please read this volume so that you can use the system for an extended period of time.

1 Software Setup 1-1

1-1 Re-setup or Updating of the Software 1-1
1-2 New Setup of the Software 1-3
1-3 Setting the System Configuration 1-5
1-3-1 Overall Setting of FLUOVIEW 1-5
1-3-2 Setting the [Microscope Control Panel] 1-15
1-4 Adding the dyeing method 1-22
1-5 Adding the filters 1-25
1-5-1 Adding Filters 1-25
1-5-2 Setting the Scanning Unit 1-26
1-5-3 Adding a Dyeing Method 1-28
1-5-4 Associating a Detection Channel and Filter to the Dyeing Method 1-30

2 Maintenance of Major System Units 2-1

2-1 Laser Scanning Microscope 2-1
2-2 UV-Ar Laser 2-2
2-2-1 Auto Alignment Operation (Remote Switch) 2-2
2-2-2 Checking, Refilling and Replacing Water in Chiller 2-3

3 Setting the Confocal Aperture 3-1

3-1 Starting the FLUOVIEW Software 3-2
3-2 Displaying the [C.A. Adjustment] Dialog Box 3-3
3-3 Confocal Aperture Setting 3-5

1 Software Setup

The FLUOVIEW software has been set up before it is delivered to the user.

A CD-ROM containing the software program is provided with FLUOVIEW. This CD-ROM is intended for use when re-setup is required due to a fault or when the user wants to set up the software newly.

When the FLUOVIEW software is updated, the user is also requested to update the software in use accordingly. For re-setup or updating of the software, see section 1-1, “Re-Setup or Updating of the Software” below and follow the procedures given in it.

When you want to set up the software newly, see section 1-2, “New Setup of the Software” below and follow the procedures given in it.

TIP

To set up the FLUOVIEW software, it is necessary that the computer in use already has Microsoft Windows XP Professional (English version) or Microsoft Windows 2000 (English version) installed in its hard disk.

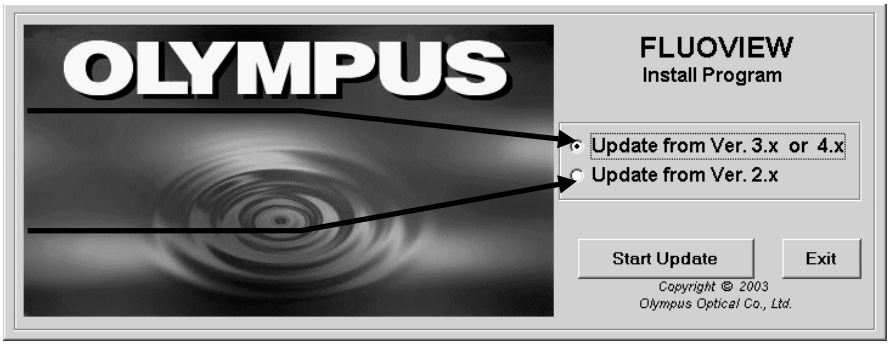
A vacant space of 80 GB is required for the setup.

1-1 Re-setup or Updating of the Software

1. Insert the FLUOVIEW setup CD-ROM in the CD-ROM drive.
   Then the dialog box as shown below appears.

[Update from Ver.3.x] option button
When updating from FV software after ver3.0, select this option button.

[Update from Ver.2.x] option button
When updating from FV software of ver2. x, press this option button.



TIP

If the dialog box doesn’t appear, run the 'Install.exe' file that is present in the root directory of the CD-ROM.

2. Click the option button of the version of FV software installed, and click the <Start Update> button.

3. [Update FV Software] dialog box is displayed and starts the current settings.
   For save, carry out according to a wizard.

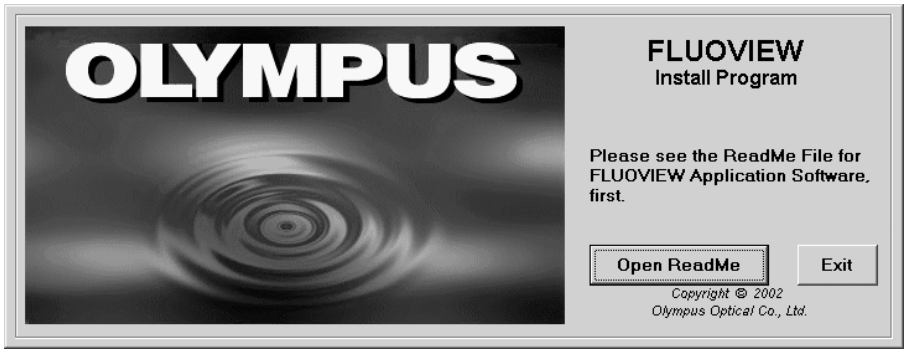
4. Continuously, deletion of software is started.
   For deletion, carry out according to a wizard.

5. Continuously, installation of the software of a new version is started.
   Refer to 1-2 “New Setup of the Software” for details.

1-2 New Setup of the Software

The following procedure allows you to set up the FLUOVIEW software in a computer you use.

1. Load the FLUOVIEW setup CD-ROM in the CD-ROM drive.
   The dialog box as shown below will appear.

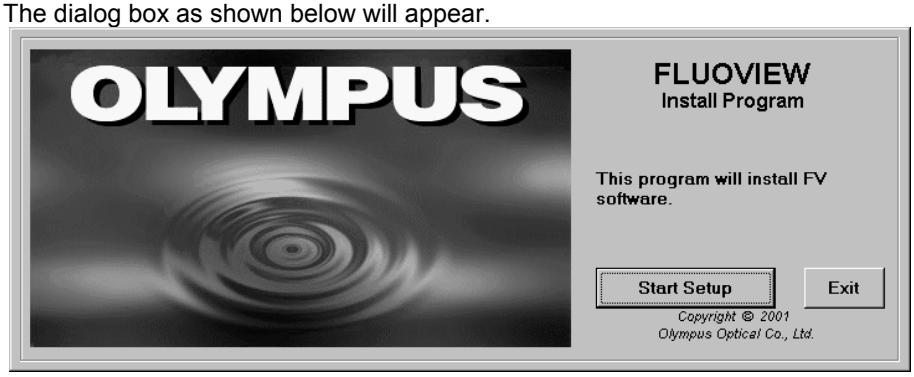


TIP If the dialog box doesn't appear, run the 'Install.exe' file that is present in the root directory of the CD-ROM.

2. Select the <Open Readme> button.

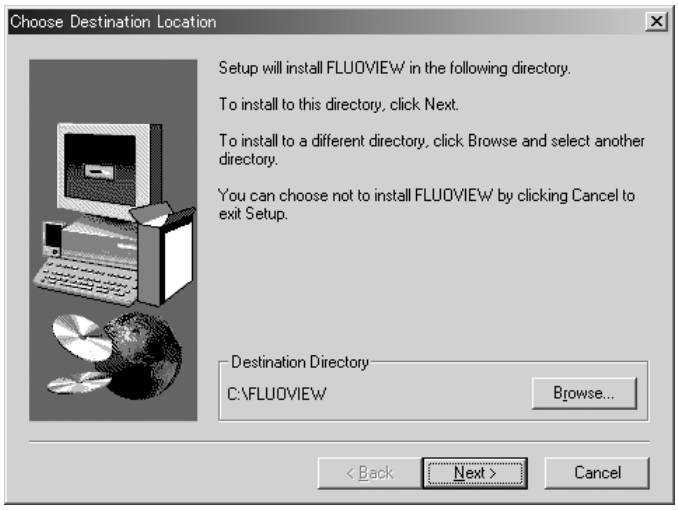
<Close> button

3. When Readme.txt is displayed, read it carefully. Click the <Close> button on the top left of the window to close Readme.txt.
   The dialog box as shown below will appear.

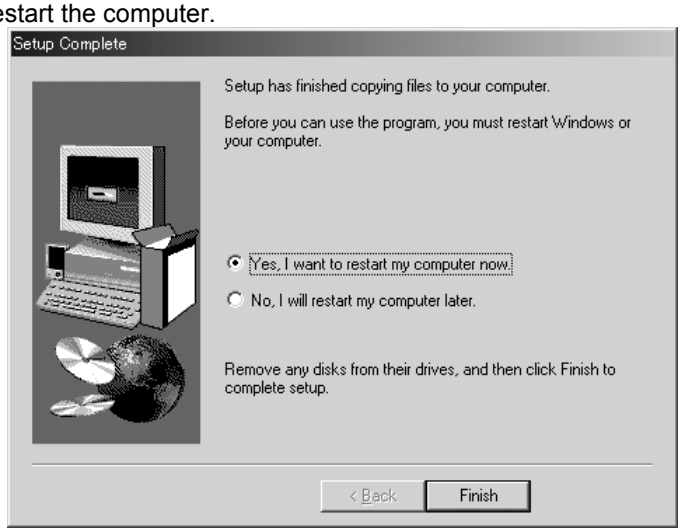


4. Select the <Start Setup> button.

5. When the [Choose Destination Location] dialog box appears, confirm the setup destination drive name and directory and select the <Next> button.


Choose Destination Location
Setup will install FLUOVIEW in the following directory.
To install to this directory, click Next.
To install to a different directory, click Browse and select another directory.
You can choose not to install FLUOVIEW by clicking Cancel to exit Setup.
Destination Directory
C:\FLUOVIEW
Browse...
< Back
Next >
Cancel

6. When the setup has completed, the [Setup Complete] dialog box appears. Select the [Yes, I want to restart my computer now.] option button and press the <Finish> button. This will restart the computer.


Setup Complete
Setup has finished copying files to your computer.
Before you can use the program, you must restart Windows or your computer.
Yes, I want to restart my computer now.
No, I will restart my computer later.
Remove any disks from their drives, and then click Finish to complete setup.
< Back
Finish

1-3 Setting the System Configuration

If it is required to change the settings after having set up the software, perform the following procedure.

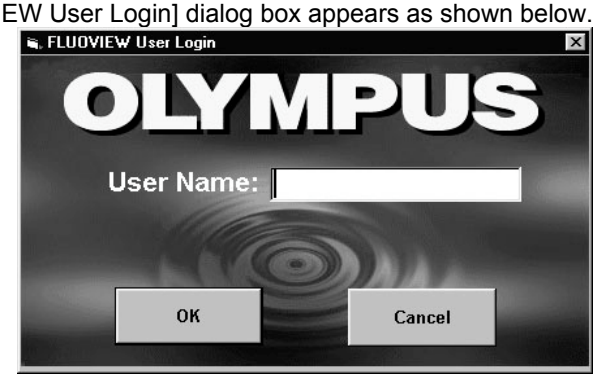
NOTE

Don't double click the [FLUOVIEW Setup] icon on the desktop and boot it up to change the system setting while the FLUOVIEW software is running. You can change the practicable setting by selecting [Tools] > [User Preference].

1-3-1 Overall Setting of FLUOVIEW

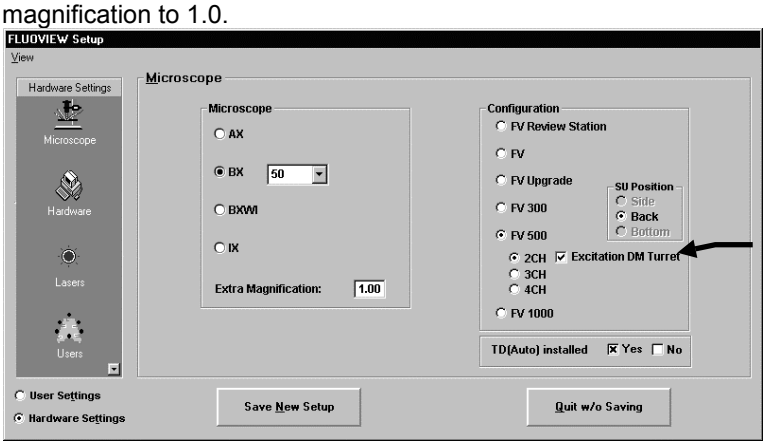
[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] on the desktop.
   The [FLUOVIEW User Login] dialog box appears as shown below.

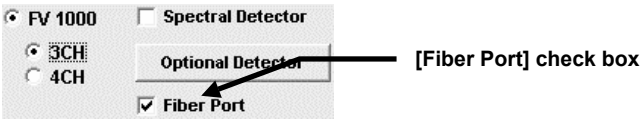


2. Enter the user name in the [User Name :] text box and click the <OK> button to log into FLUOVIEW FV1000.
   Refer to Appendix H-2 "Logging into the FV1000" for details.

3. The dialog as shown below appears for use in saving the system configuration.
   First, select the type of microscope and set the extra (intermediate attachment) magnification to 1.0.



[Excitation DM Turret] check box
Check box appears when selecting “2CH”. Check when using a varialbe excitaion dichroic mirror.

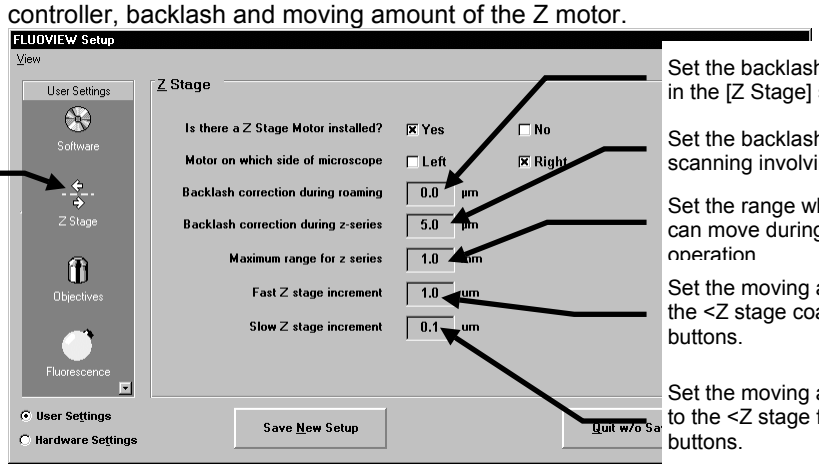


[Fiber Port] check box

When the channel 3 of the FV1000 is selected, the [Fiber Port] check box will be available. Please check here when a fiber port is connected to the FLUOVIEW.

Selecting “IX71” and “Automatic” in the [Microscope] group box or “BX61, 62”, “BX61, 62WI”, or “IX81” in the [Microscope] group box shows the [Microscope Setup] dialog box to edit the BX operation panel (BX Control Panel). For details, see section 1-3-2 “Setting the [Microscope Control Panel]”.

4. Click the [Z Stage] icon in [User Settings] to display the [Z Stage] panel in the front position, and check and set the presence of motor controller, positioning of the motor controller, backlash and moving amount of the Z motor.

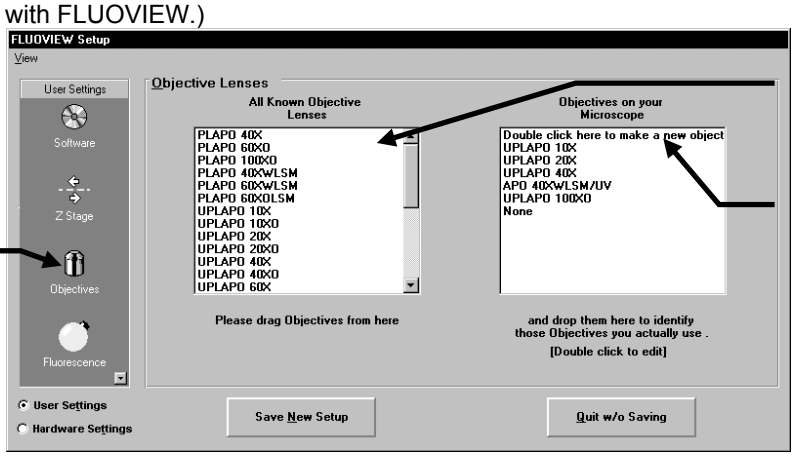


TIP

The [Maximum range for Z series (mm)] text box accepts a setting value between 1.0 and 3.0 (mm).
The values in the [Start Z] and [Stop Z] text boxes in the [Z Stage] sub-panel are set according to the value set there.
For example:
When “1.0” (mm) is set as the maximum range for Z series, the minimum value for Z starting becomes -500 (μm) and the maximum value for Z stopping becomes 500 (μm).

5. Click the [Objectives] icon in [User Settings] to display the [Objectives] panel in the front position.

   In this panel, select the items to be included in the list of objectives used by the FLUOVIEW application. (Each user should set the objectives that the user wants to use with FLUOVIEW.)



[Objectives] icon

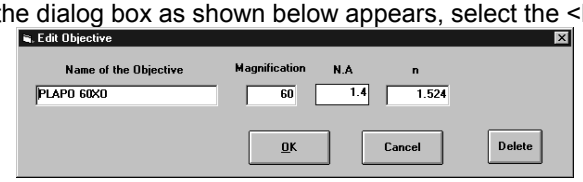
List of all objectives available at present

List of objectives that can be used during FLUOVIEW operation

<To delete an unnecessary objective from the list>

In the list on the right, double-click the objective to be deleted from the list.

When the dialog box as shown below appears, select the <Delete> button.



<To change the details (objective name, pinhole No., magnification) of an objective>

Double-click the objective for which you want to change the detailed information. When the dialog box appears, change the desired information items and select the <OK> button.

<To add an objective to the list>

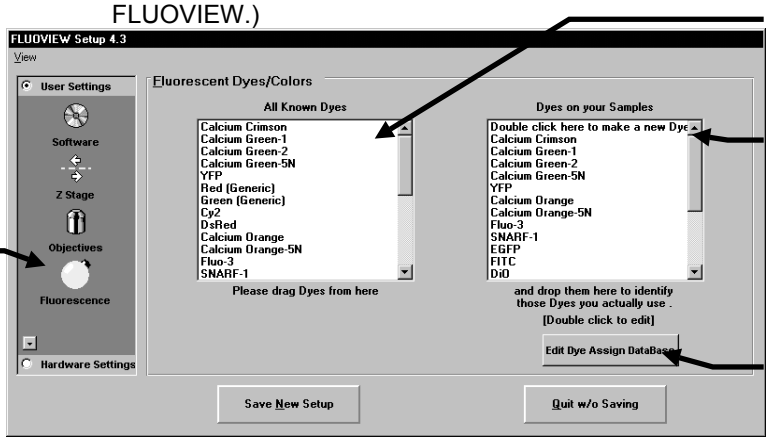
Double-click “Double Click here...” at the top of the list on the right. When the dialog box appears, set the detailed information on the new objective and select the <OK> button.

<To add one of the objectives in the list on the left to the list on the right>

Place the mouse pointer on the objective to be added in the list on the left, and drag it to the list on the right.

6. Click the [Fluorescence] icon in [User Settings] to display the [Fluorescent Deys/Colors] panel in the front position.

   In this panel, select the items to be included in the list of dyes used by the FLUOVIEW application. (Each user should set the dyes that the user wants to use with FLUOVIEW.)



List of all dyes available at present

List of dyes that can be used during FLUOVIEW operation

[Fluorescence] icon

<Edit Dye Assign Database> button

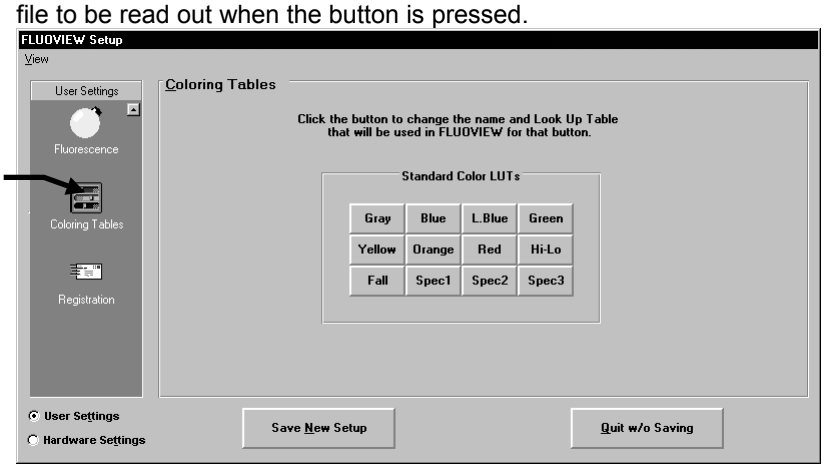
By clicking this button, [Optical System Configuration Setup] appears to configure image acquisition channel, filter, dyeing method.

For details, see section 1-5-4 of this manual.

The setting procedure is similar to the objective setting procedure ([Objectives] panel).

7. Click the [Coloring Tables] icon in [User Settings] to display the [Coloring Tables] panel in the front position.

   In this panel, select the characters to be displayed in each of the 8 buttons displayed in the [Color Tool] dialog box of the FLUOVIEW application and the LUT file to be read out when the button is pressed.

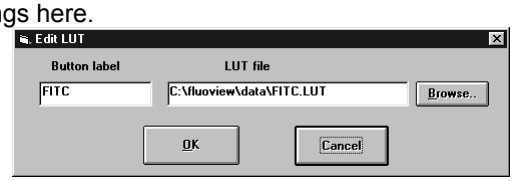


[Coloring Tables] icon

<To change the button settings>

Press the button to indicate that you want to change the setting. When the dialog box as shown below appears, specify the button name and LUT file name and select the <OK> button.

The LUT files which have been used up to Ver 1.0* (FITC, etc.) are supplied with this version in the same names as before. If you want to use such files, change their settings here.



8. Click the [Software] icon in [User Settings] to display the [Software] panel in the front position.

   In this panel, select the initial magnification status when images are to be displayed in image windows. This panel is also used to set the mode of comment overlay in image and to set the numbers of Function window and image window that can be displayed together and enable/disable the automatic black-level adjustment.

[Coloring for new overlays] group box

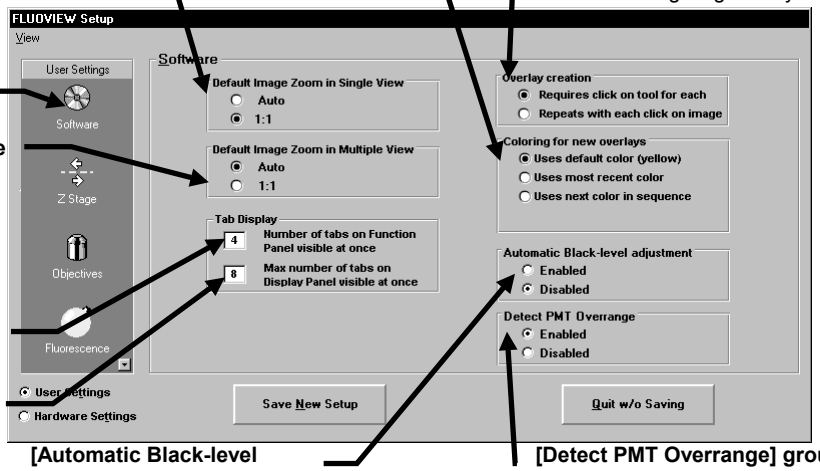
Set the color of the comment to be overlaid in image in the image window. [Uses next color in sequence] changes the color of the comments sequentially.

[Overlay creation] group box

When comment is to be overlaid in image, select either the mode allowing repeated entries of comment per Annotation tool selection or the mode allowing single entry of comment.

[Default Image Zoom in Single View] group box

Select the initial status of display magnification when a single image is to be displayed in a single image window.



[Software] icon

[Default Image Zoom in Multiple View] group box

Select the initial status of display magnification when multiple images are to be displayed by displaying multiple image window together.

Set the number of Function windows that can be displayed at a time.

Set the number of image windows that can be displayed at a time.

[Automatic Black-level adjustment] group box

Select Enabled/Disabled to enable/disable the automatic black-level adjustment.

[Detect PMT Overrange] group box

Select [Enabled]/[Disabled] to enable/disable display the message which notifies that the Output excessive light to PMT.

9. Click the [Hardware] icon in [Hardware Settings] to display the [Hardware] panel in the front position.

   In this panel, enter the values for delay timing for TD unit and enter the maximum duration for continuous bi-directional scanning when setting fast scan mode.

[Delay Timing for TD Unit] group box

This data should usually be left in the default values and does not have to be changed.

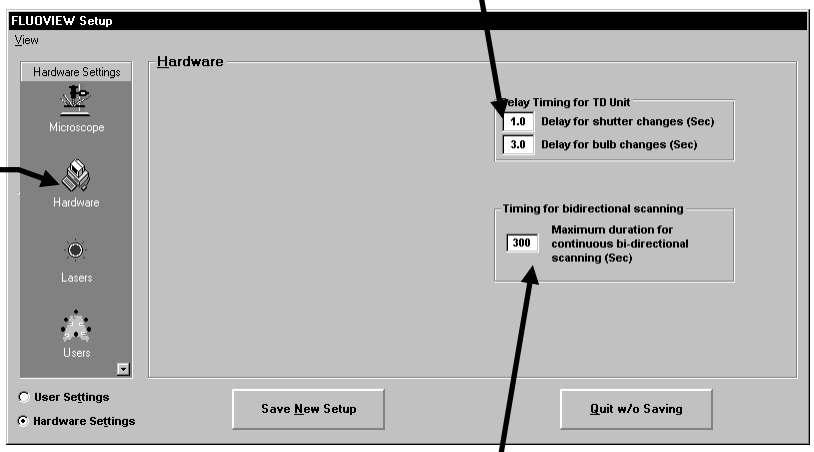
[Delay shutter changes (sec)] text box

Enter the opening/closing wait time of the shutter in the TD unit in seconds.

[Delay for bulb changes (sec)] text box

Enter the ON/OFF wait time of the lamp in the TD unit in seconds.

[Hardware] icon



[Timing for bidirectional scanning] group box

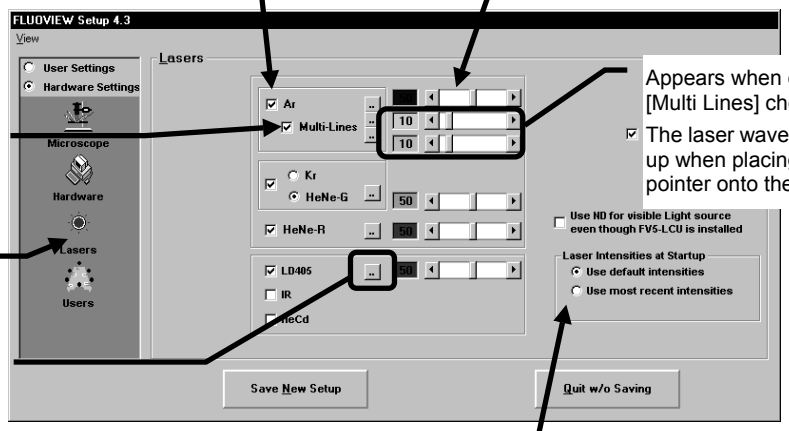
Enter the maximum duration for continuous bi-directional scanning (in seconds) when setting fast scan mode.

10. Click the [Lasers] icon in [Hardware Settings] to display the [Lasers] panel in the front of position.

    Set the lasers to be used and set its default intensity.

[Lasers Installed] check box
Select and check the lasers to be used with the FLUOVIEW application.

[Default Laser Intensity] scale
Set the initial ND filter value at the start of the FLUOVIEW application.



[Multi Lines] check box
Check when using Multiline Ar laser, then the scale appears to the right

[Lasers] icon

[Detail Setting] button

Appears when checking the [Multi Lines] check box.

The laser wave length is popped up when placing the mouse pointer onto the text box.

[Laser Intensities at Startup] group box
Set the laser intensities when starting up the FLUOVIEW application.

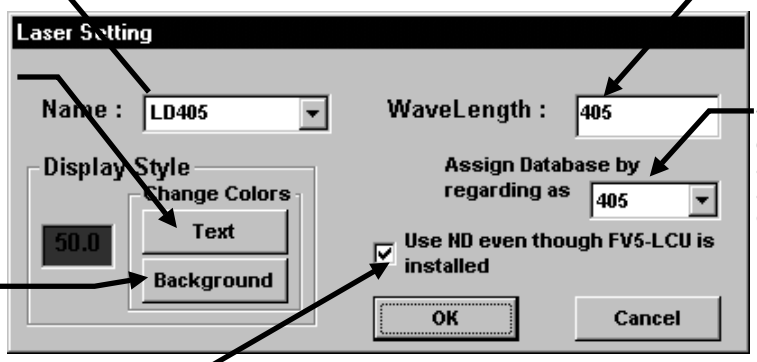
[User default intensities] option button
Select to use the default intensity set in the [Default Laser Intensity] scale above.

[Use most recent intensities] Option button
Set to use the intensity that was used in the last observation.

11. Click the [Detail Setting] button to display [Laser Setting] dialog box.

[Name] drop down list
Select laser name. The name can be also input from the keyboard.

[Text] button
Set color of characters to display laser intensity.



Input laser wavelength to be displayed in pop up dialog in laser intensity display.

This value is utilized to determine filter setting in accordance with dye, and it does not need to change usually.

[Background] button
Set background color of laser intensity display.

Check this box if dedicated AOTF is not equipped to the laser unit selected from drop down list of [Name].

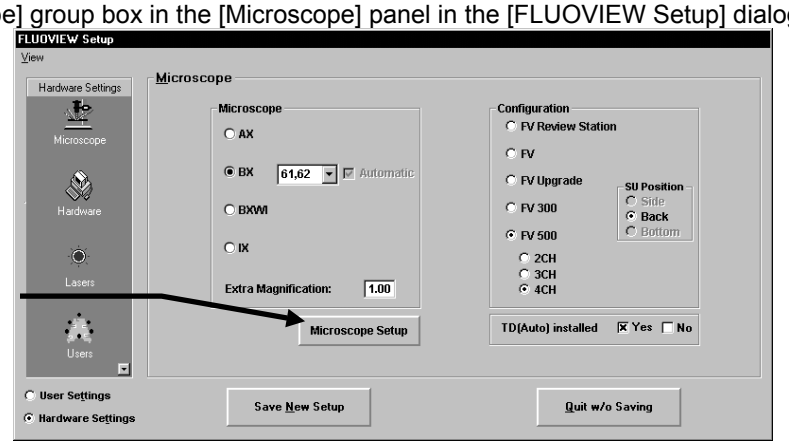
12. The [Registration] panel have been set at the factory and do not need to be changed here.

13. After completing the setup, select the <Save New Setup> button on the bottom left of the panel.
    (Selecting <Quit w/o Saving> exits the panel without saving the system setup.)

1-3-2 Setting the [Microscope Control Panel]

The <Microscope Setting> button as shown below appears when selecting “IX71” and checking the [Automatic] check box or selecting “BX61, 62”, “BX61,62WI”, or “IX81” in the [Microscope] group box in the [Microscope] panel in the [FLUOVIEW Setup] dialog box.

<Microscope Setup> button
Displays the [Microscope Setup] window.



Clicking the <Microscope Setup> button displays the [Microscope Setup] window as shown below.

[Filter Turret] group box
Sets up the name and color of the filter turret for reflected light observation.

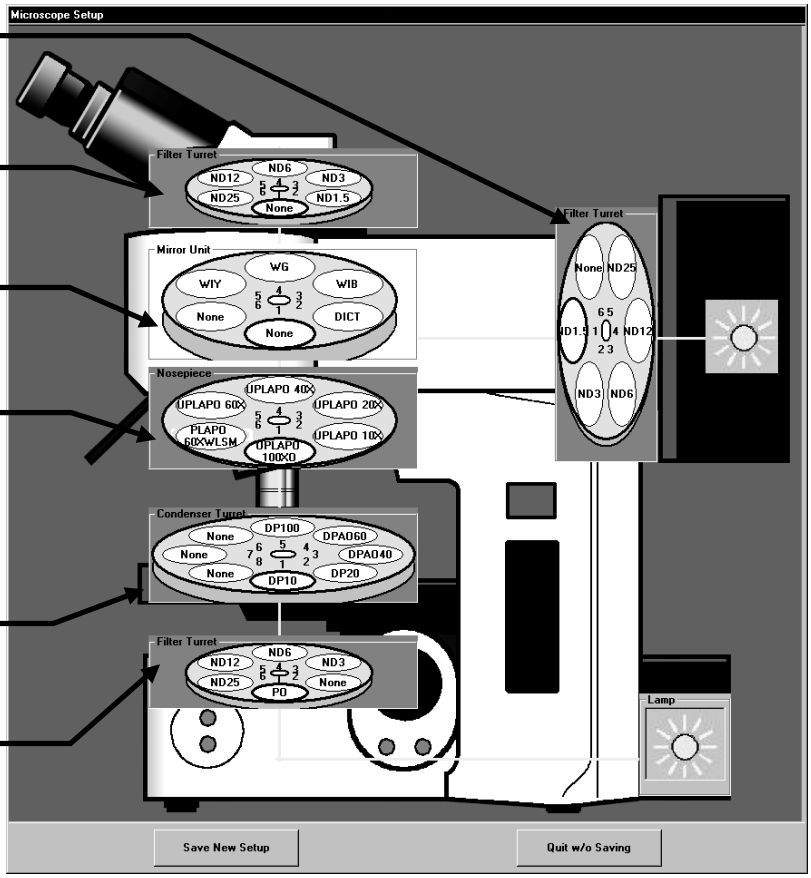
[Filter Turret] group box
Sets up the name and color of the filter turret for visual observation.

[Mirror Unit] group box
Sets up the name and color of the cube turret.

[Nosepiece] group box
Sets up the name, color of the objective and sets the magnification, N.A, refractive index (n), number of the confocal pinhole, and the condenser worked with to each objective.

[Condenser Turret] group box
Sets up the name and color of the universal condenser.

[Filter Turret] group box
Sets up the name and color of the filter turret for transmitted observation.



(Combination with BX)

[Shutter] group box

Sets up to use the shutter for transmitted observation.

[Filter Turret] group box

Sets up the name and color of the filter turret for transmitted observation.

[Condenser Turret] group box

Sets up the name and color of the universal condenser.

[Nosepiece] group box

Sets up the name, color of the objective and sets the magnification, N.A, refractive index (n), number of the confocal pinhole, and the condenser worked with to each objective.

[Filter Turret] group box

Sets up the name and color of the filter turret for visual observation.

[Mirror Unit] group box

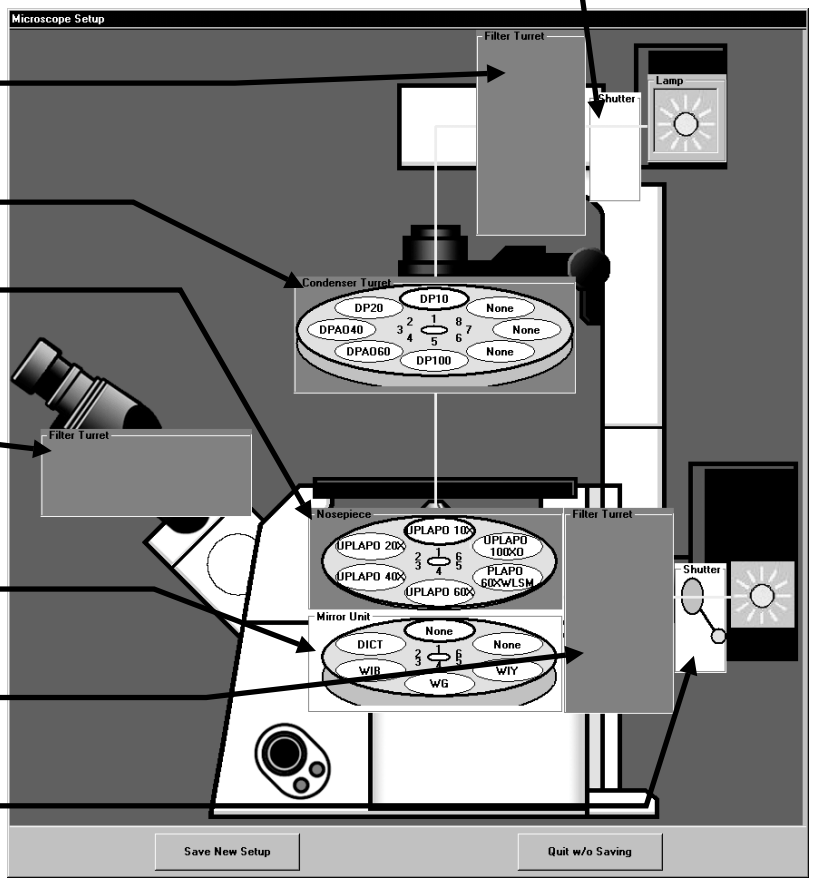
Sets up the name and color of the cube turret.

[Filter Turret] group box

Sets up the name and color of the filter turret for reflected light observation.

[Shutter] group box

Sets up to use the shutter for reflected light observation.

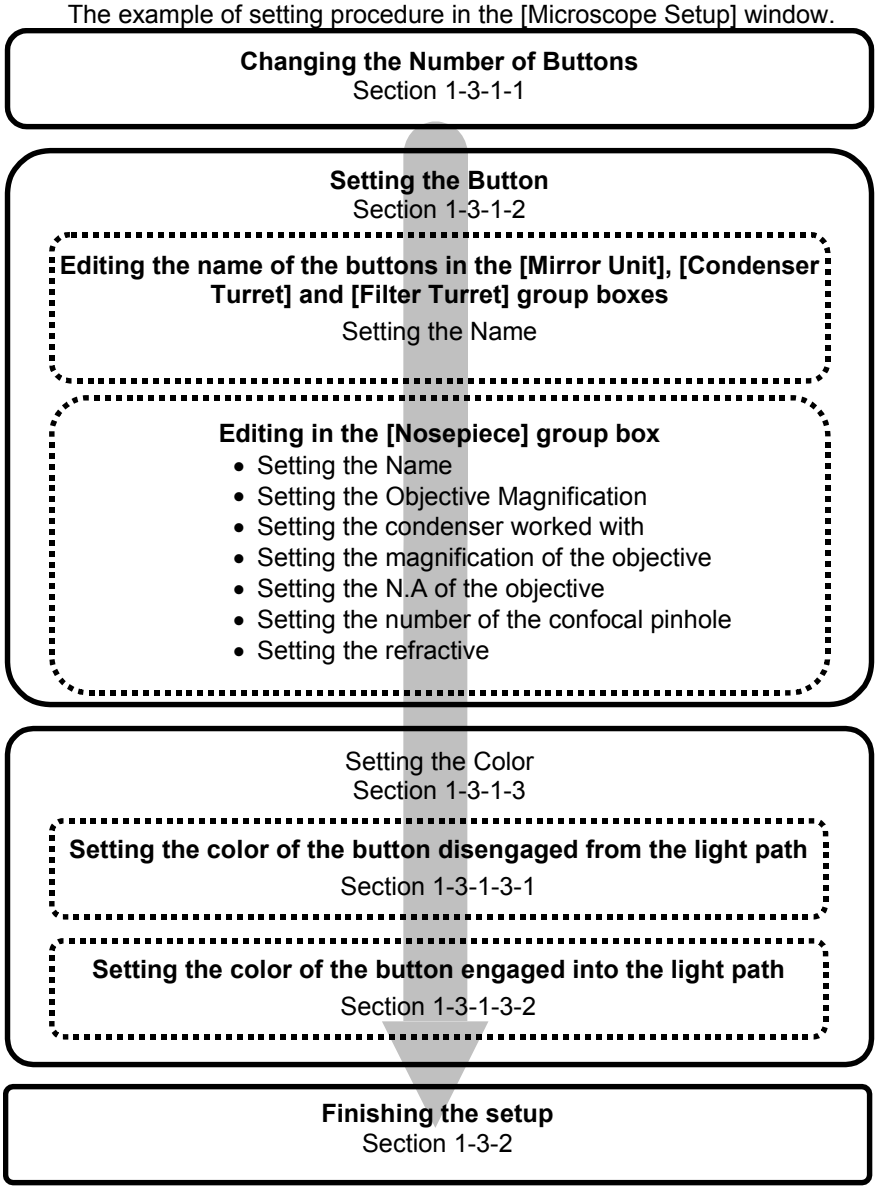


(Combination with IX)

NOTE

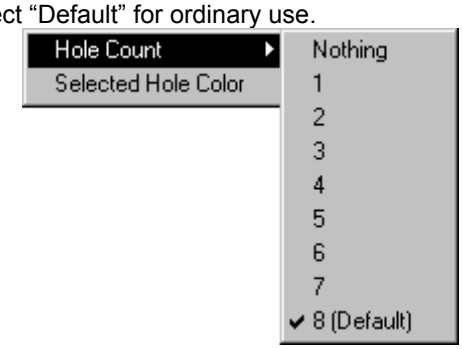
When using IX, three parts can be motorized among the filter turrets, the mirror unit, and condenser turret.

The example of setting procedure in the [Microscope Setup] window.


Changing the Number of Buttons
Section 1-3-1-1
Setting the Button
Section 1-3-1-2
Editing the name of the buttons in the [Mirror Unit], [Condenser Turret] and [Filter Turret] group boxes
Setting the Name
Editing in the [Nosepiece] group box
Setting the Name
Setting the Objective Magnification
Setting the condenser worked with
Setting the magnification of the objective
Setting the N.A of the objective
Setting the number of the confocal pinhole
Setting the refractive
Setting the Color
Section 1-3-1-3
Setting the color of the button disengaged from the light path
Section 1-3-1-3-1
Setting the color of the button engaged into the light path
Section 1-3-1-3-2
Finishing the setup
Section 1-3-2

1-3-2-1 Setting the Number of Buttons

1. Right-click the mouse on the area outside of the buttons in the group box where the number of buttons to be displayed is changed. The pop-up menu as shown below appears. Select “Hole Count” and select the number of the buttons to be displayed in the sub-menu. Select “Default” for ordinary use.

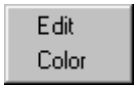


TIP Selecting “Nothing” disables the turret itself.

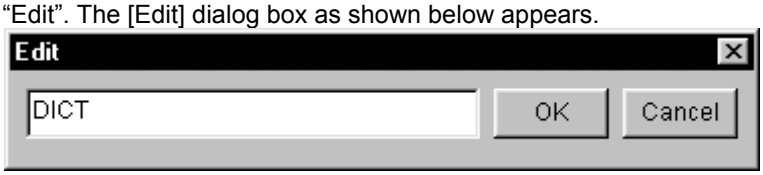
1-3-2-2 Setting the Name

1 Editing the name of the buttons in the [Mirror Unit], [Condenser Turret] and [Filter Turret] group boxes

1. In the [Mirror Unit], [Condenser Turret] or [Filter Turret] group box, right-click the mouse on the button whose name is to be edited. The pop-up menu as shown below appears.



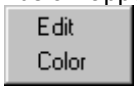
2. Select “Edit”. The [Edit] dialog box as shown below appears.



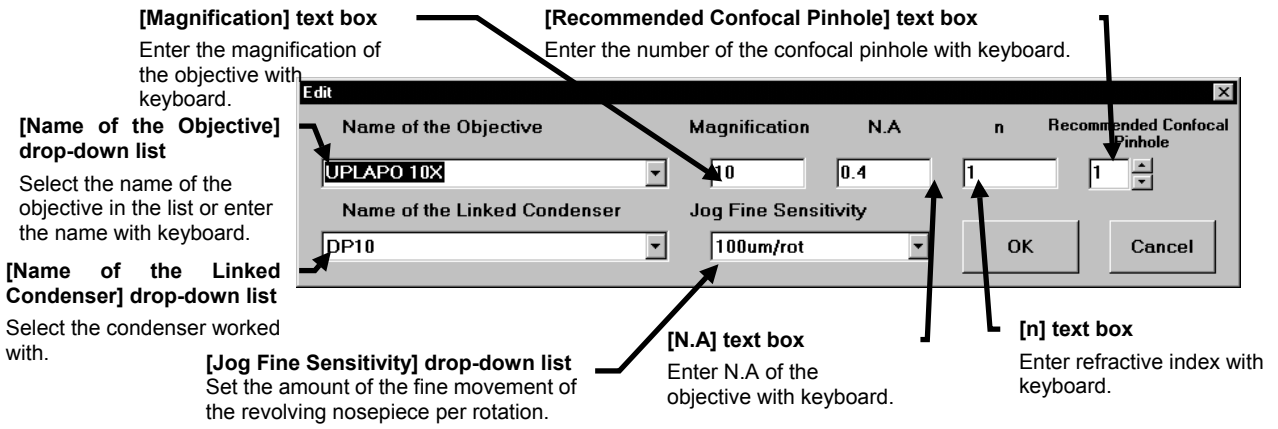
3. Enter a name into the text box and click the <OK> button.

2 Editing in the [Nosepiece] group box

1. In the [Nosepiece] group box, right-click the mouse on the button whose name is to be edited. The pop-up menu as shown below appears.



2. Select “Edit”. The [Edit] dialog box as shown below appears.


[Magnification] text box
Enter the magnification of the objective with keyboard.
[Recommended Confocal Pinhole] text box
Enter the number of the confocal pinhole with keyboard.
Edit
Name of the Objective
Magnification
N.A
n
Recommended Confocal Pinhole
UPLAPO 10X
10
0.4
1
1
Name of the Linked Condenser
Jog Fine Sensitivity
DP10
100um/rot
OK
Cancel
[Name of the Objective] drop-down list
Select the name of the objective in the list or enter the name with keyboard.
[Name of the Linked Condenser] drop-down list
Select the condenser worked with.
[Jog Fine Sensitivity] drop-down list
Set the amount of the fine movement of the revolving nosepiece per rotation.
[N.A] text box
Enter N.A of the objective with keyboard.
[n] text box
Enter refractive index with keyboard.

3. Click the <OK> button after setting is completed.

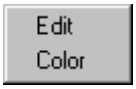
TIP

The initial values are automatically set in the [Magnification], [N.A], [n], and [Recommended Cofocal Pinhole] text boxes after the objective is selected in the [Name of the Objective] drop-down list.

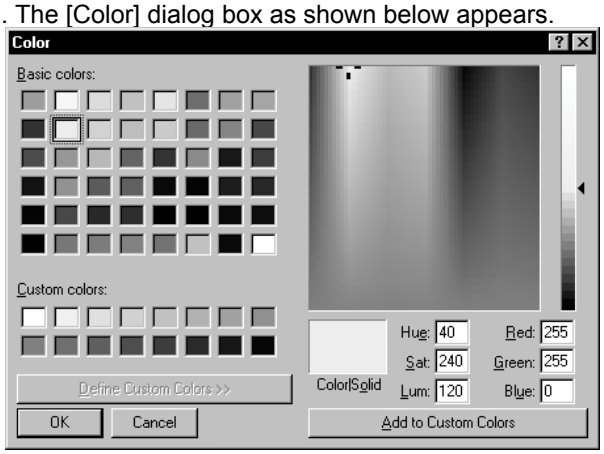
1-3-2-3 Setting the Color

1 Setting the color of the button disengaged from the light path

1. Right-click the mouse on the button whose color is to be changed. The pop-up menu as shown below appears.



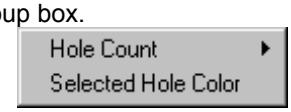
2. Select “Color”. The [Color] dialog box as shown below appears.



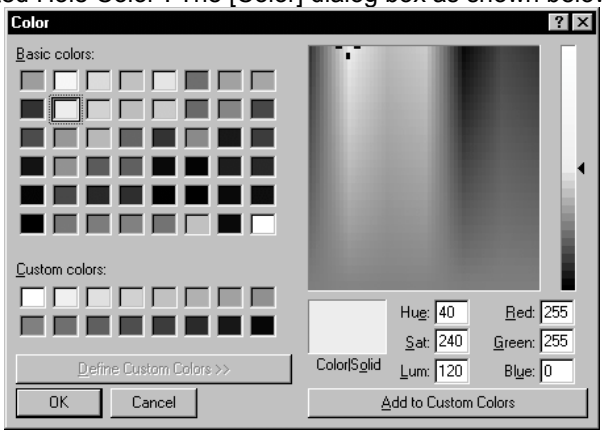
3. Select the color which you want to set in the color palette and click the <OK> button.

2 Setting the color of the button engaged into the light path

1. In [BX Control Panel] window on the software, specify the color of the button of the cube, the objective, and the condenser engaged into the light path. Right-click the mouse on the area outside of the buttons in the [Mirror Unit], [Nosepiece], [Condenser Turret], or [Filter Turret] group box.



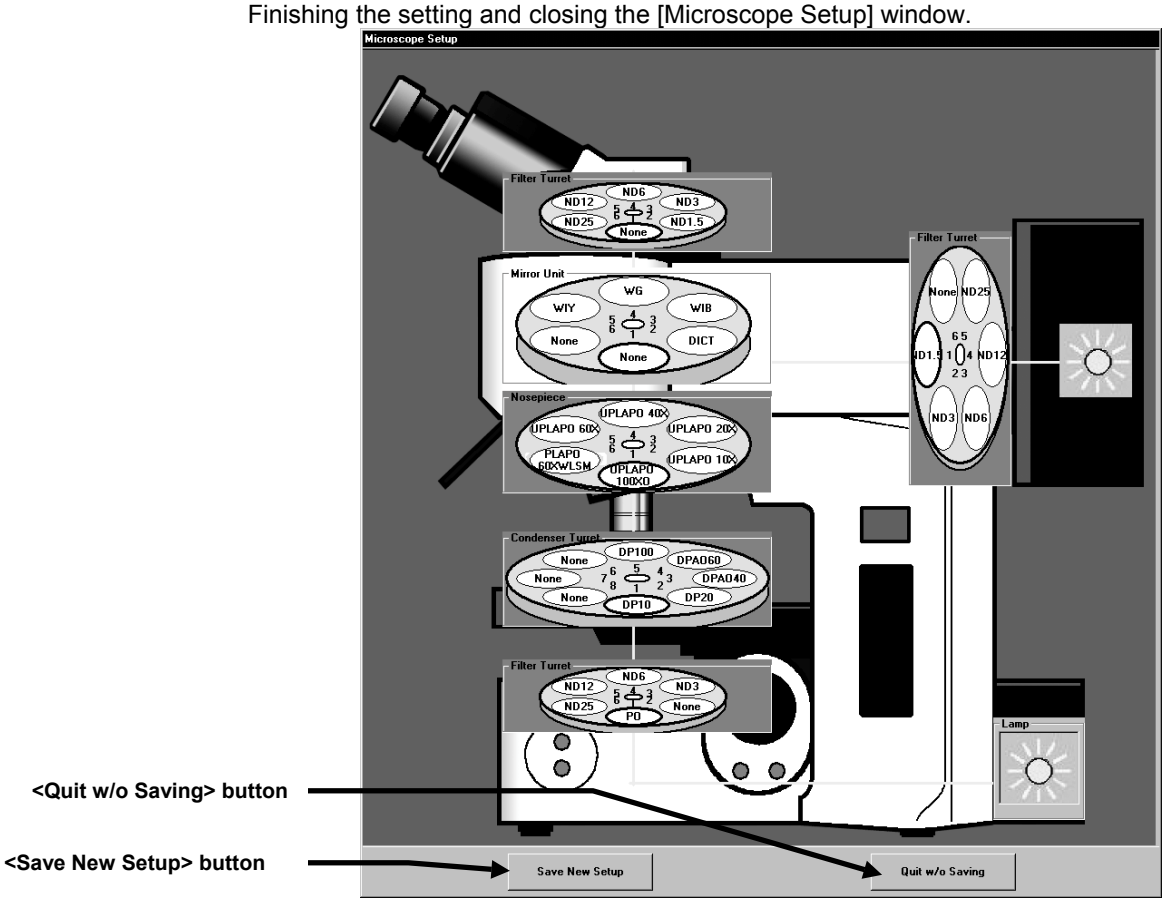
2. Select “Selected Hole Color”. The [Color] dialog box as shown below appears.



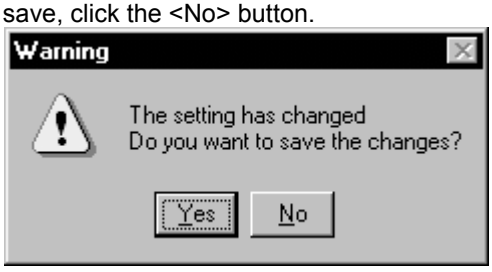
3. Select the color which you want to set in the color palette and click the <OK> button.

1-3-2-4 Finishing the setting

Finishing the setting and closing the [Microscope Setup] window.



1. Click the < Save New Setup> button to save the setup and close the window.
   Or click the <Quit w/o Saving> button to cancel the setup and close the window. Then the dialog box as shown below appears. If you want to save, click the <Yes> button, if you do not want to save, click the <No> button.



NOTE **After saving is selected in the [Microscope Setting] window, the original setting is not returned even if the <Quit w/o Saving> is clicked in the [FLUOVIEW Setup] dialog box.**

1-4 Adding the dyeing method

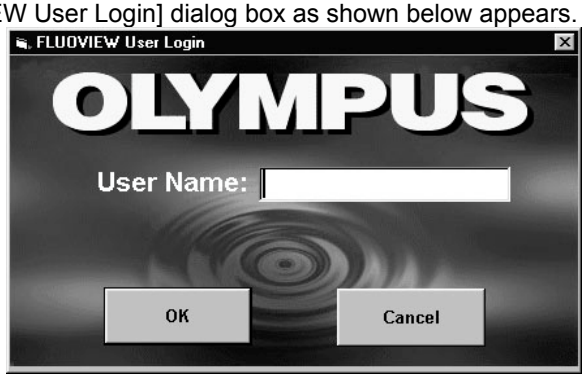
Newly add a dyeing method and set laser type, excitation wavelength, and emission wavelength.

This section describes a simple example of adding CFP as a dyeing method and setting laser type, excitation wavelength, and emission wavelength.

Add the dyeing method using the [Fluorescent Dyes/Colors] panel in the [Fluoview Setup] dialog box.

[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.
   The [FLUOVIEW User Login] dialog box as shown below appears.

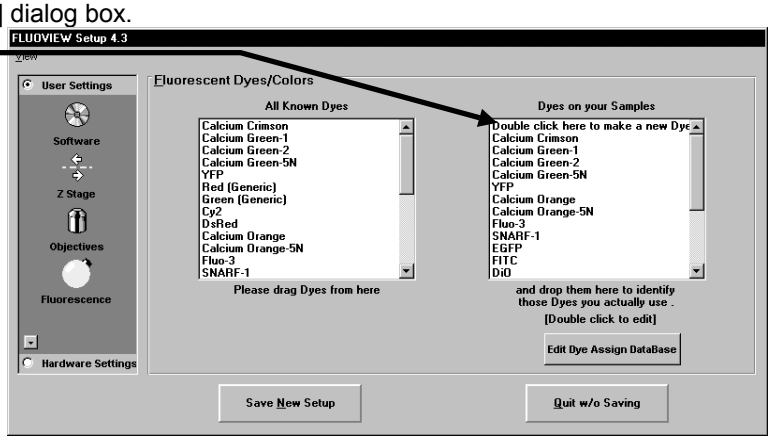


2. Enter user name into the [User Name:] text box and click the <OK> button to log into FLUOVIEW FV1000.

TIP When using the system for one user, the [FLUOVIEW User Login] dialog box does not appear.

3. Display the [Fluorescent Dyes/Colors] panel at the front position in the [FLUOVIEW Setup] dialog box.

Double-click here to add the dyeing method.

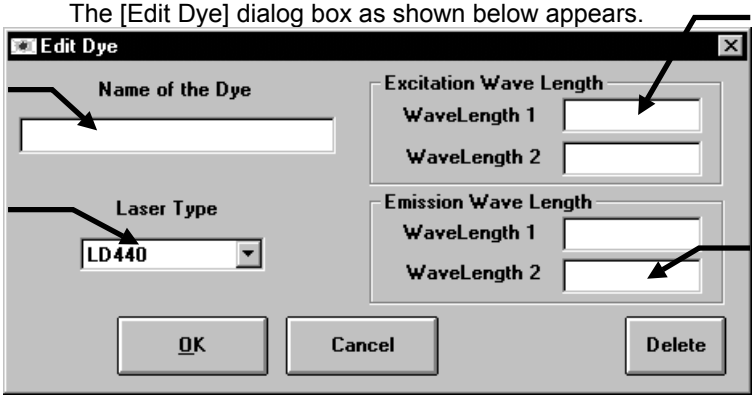


4. Double-click the “Double click here to make a new Dye” in the [Dyes on your Samples] list.

   The [Edit Dye] dialog box as shown below appears.

[[Name of the Dye] text box
Enter the name of the dyeing method.

[Laser Type] drop-down list
Select the laser to use.



[Excitation Wave Length] group box
Enter the excitation wavelength. In case of the 2-wavelength and 1-photometry, enter the value into the [[WaveLength2] text box

[Emission Wave Length] group box
Enter the emission wavelength.
In case of the 1-wavelength and 2-photometry, enter the value into the [[WaveLength2] text box too.

5. Enter the name of the dyeing method into the [Name of the Dye] text box (e,g, CFP).

6. Select the laser to use using the [Laser Type] drop-down list (e.g. LD440).

TIP

As the laser to use, LD440 laser should be set in advance. When “LD440” is not displayed, check the [LD440] check box in the [Laser Equipment] panel.

7. Enter the value of the excitation wavelength into the [WaveLength 1] text box in the [Excitation Wave Length] group box (e.g. 442).

8. Enter the value of the emission wavelength into the [WaveLength 1] text box in the [Emission Wave Length] group box (e.g. 480).

TIP

photometry, enter the value of the wavelength into the [WaveLength 2] text box too.

9. Click the <OK> button to close the [Edit Dye] dialog box.

10. Select the <Save New Setup> button to close the [FLUOVIEW Setup] dialog box.

TIP

Some dyeing method does not appear in [Available Dyes] list box in [Dyes] subpanel.
It happens when there is no relation between the dyeing method and image acquisition channel.
The relation of dye and the channel can be done by check [Assign dyes manually] check box, or see section 1-5-4, “Associating a Detection Channel and Filter to the Dyeing Method” for the setting.

1-5 Adding the filters

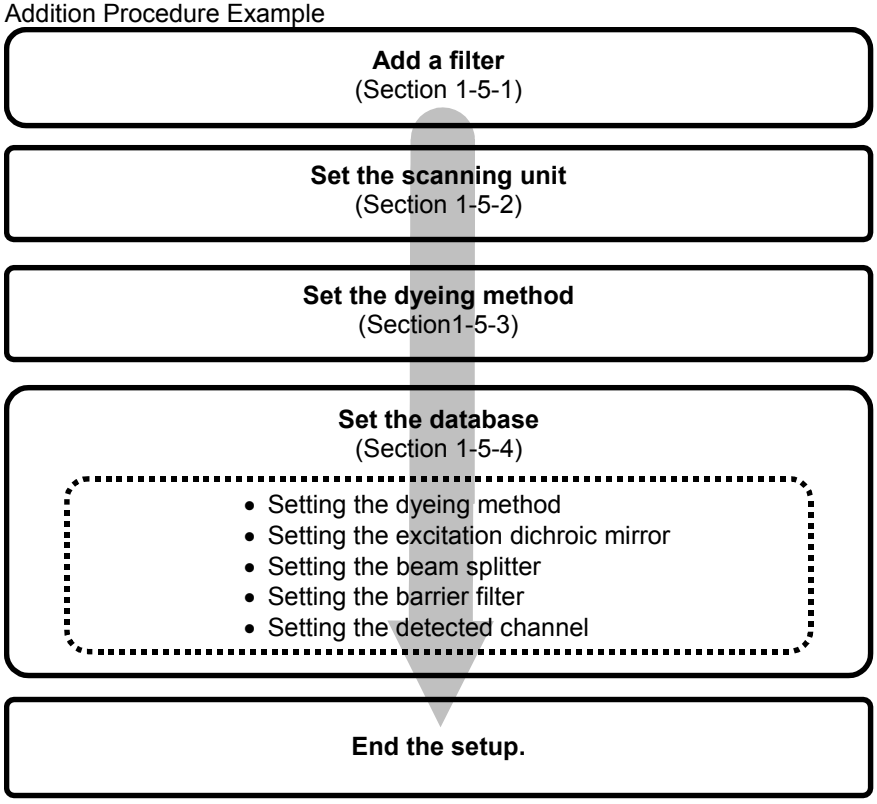
The FLUOVIEW software allows the filters to be associated with dyeing methods.

As a result, specifying a dyeing method sets the detection channel and added filter automatically.

This section assumes that a filter is added to the scanning unit and describes how to add the new filter in the software and how to associate the added filter to a dyeing method by taking the optional filter addition procedure as an example.

Example) To add “CFP” (which uses the LD440 laser) to the dyeing method and add “DM440” (optional excitation dichroic mirror) and “BA465-495” (optional barrier filter) to Channel 1 and detect “CPF” in Channel 1.

Addition Procedure Example



1-5-1 Adding Filters

For the method of adding filters to the scanning unit, please consult your local Olympus representative for assistance.

In this example, let us add “DM440” to excitation dichroic mirror turret No. 5 and ”BA465-495” to Channel 1 barrier filter No. 3.

1-5-2 Setting the Scanning Unit

Set up the scanning unit in the [Scanning Unit] panel in the [FLUOVIEW Setup] dialog box.

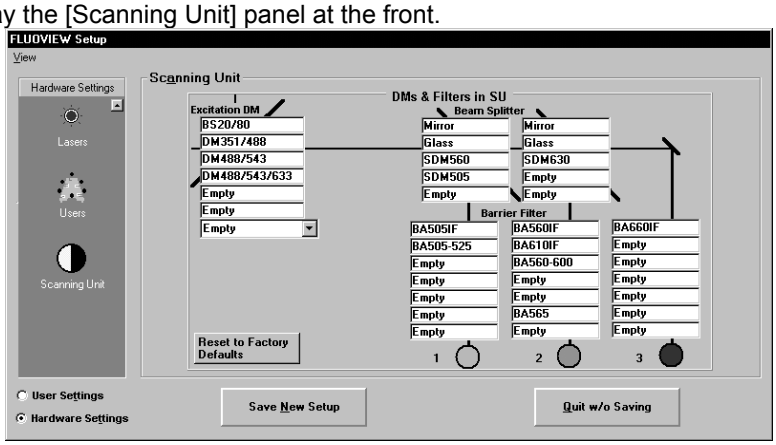
[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.
   The [FLUOVIEW User Login] dialog box shown below appears.

2. Enter “Administrator” in the [User Name:] text box and click the <OK> button to log in the FLUOVIEW FV1000.

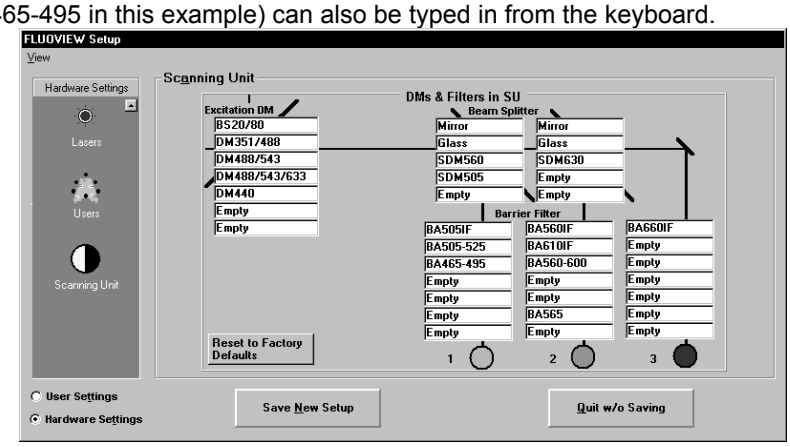
TIP The [FLUOVIEW User Login] dialog box is not displayed if the system is used in a single-user configuration.

3. Display the [Scanning Unit] panel at the front.



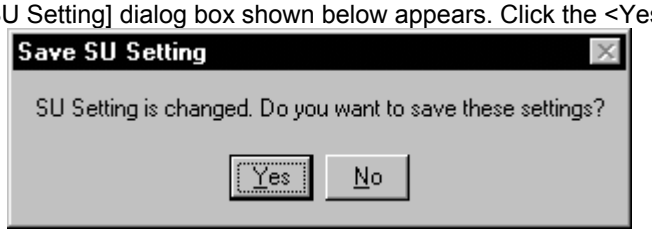
4. In the fifth field from the top of [Excitation DM] in the [DMs & Filters in SU] dialog box, select the excitation dichroic mirror name (DM440 in this example) from the drop-down list. The excitation diachronic mirror name (DM440 in this example) can also be typed in from the keyboard.

5. In Channel 1 of [Barrier Filter] in the [DMs & Filters in SU] dialog box, select the barrier filter name (BA465-495 in this example) from the drop-down list. The barrier filter name (BA465-495 in this example) can also be typed in from the keyboard.



6. Click the <Save New Setup> button.

   The [Save SU Setting] dialog box shown below appears. Click the <Yes> button.



   The [FLUOVIEW Setup] dialog box closes.

1-5-3 Adding a Dyeing Method

Add the dyeing method in the [Fluorescence] panel in the [FLUOVIEW Setup] dialog box.

[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.
   The [FLUOVIEW User Login] dialog box shown below appears.

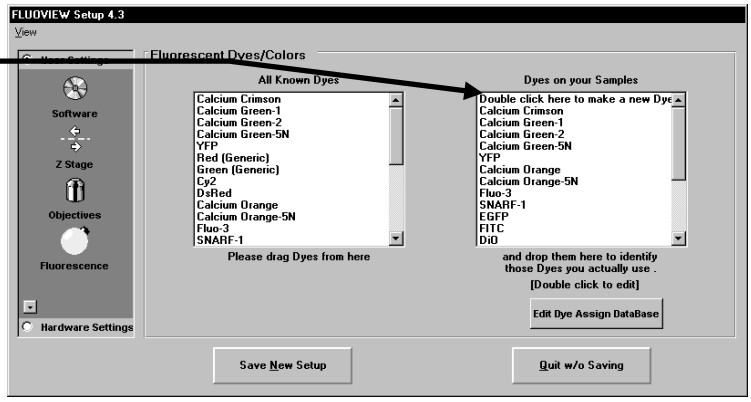
2. Enter the user name in the [User Name:] text box and click the <OK> button to log in the FLUOVIEW FV1000.

TIP

The [FLUOVIEW User Login] dialog box is not displayed if the system is used in a single-user configuration.

3. In the [FLUOVIEW Setup] window, display the [Fluorescent Dyes/Colors] panel at the front.

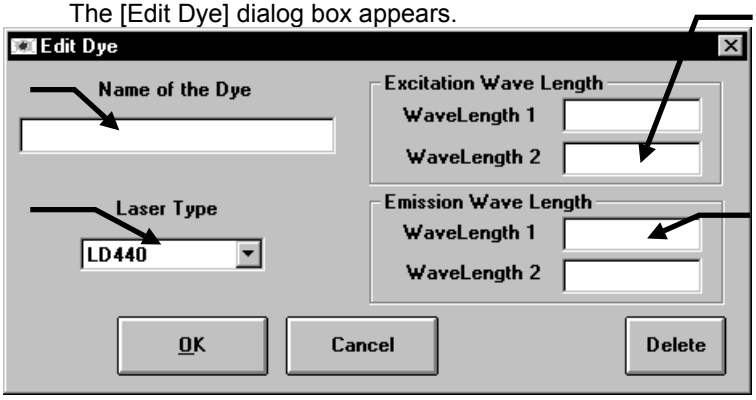
Double-click here to add a dyeing method.



4. In the [Dyes on your Samples] list, double-click “Double click here to make a new Dye”.
   The [Edit Dye] dialog box appears.

[Name of the Dye] text box
Enter the dyeing method name.

[Laser Type] drop-down list
Select the laser to be used.



[Excitation Wave Length] group box
Enter the excitation wavelength.

[Emission Wave Length] group box
Enter the emission wavelength.
In case of single excitation double emission, also enter a wavelength in the [WaveLength 2] text box.

5. Enter the dyeing method name (CFP in this example) in the [Name of the Dye] text box.

6. Select the laser to be used (LD440 in this example) from the [Laser Type] drop-down list.

TIP

It is required that the LD440 laser has been set as one of the laser types to be used in advance. If ”LD440” is not displayed, open the [Laser Equipment] panel and check the [LD440] checkbox.

7. Enter the excitation wavelength value (480 in this example) in the [WaveLength 1] text box in the [Excitation Wave Length] group box.

8. Enter the emission wavelength value (505 in this example) in the [WaveLength 1] text box in the [Emission Wave Length] group box

TIP

In case of single excitation double emission, also enter an emission wavelength in the [WaveLength 2] text box.

9. Click the <OK> button to close the [Edit Dye] dialog box.

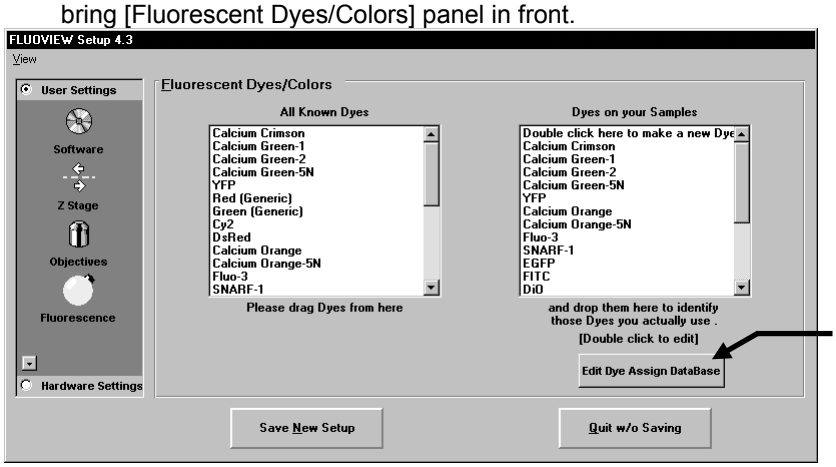
10. Click the <Save New Setup> button to close the [FLUOVIEW Setup] dialog box.

1-5-4 Associating a Detection Channel and Filter to the Dyeing Method

The detection channel and filter can be associated to a dyeing method using the [Optical System Configuration Setup] dialog box.

1. Display [FLUOVIEW Setup] dialog. See section 1-3-1 of this manual to display [FLUOVIEW Setup] dialog.

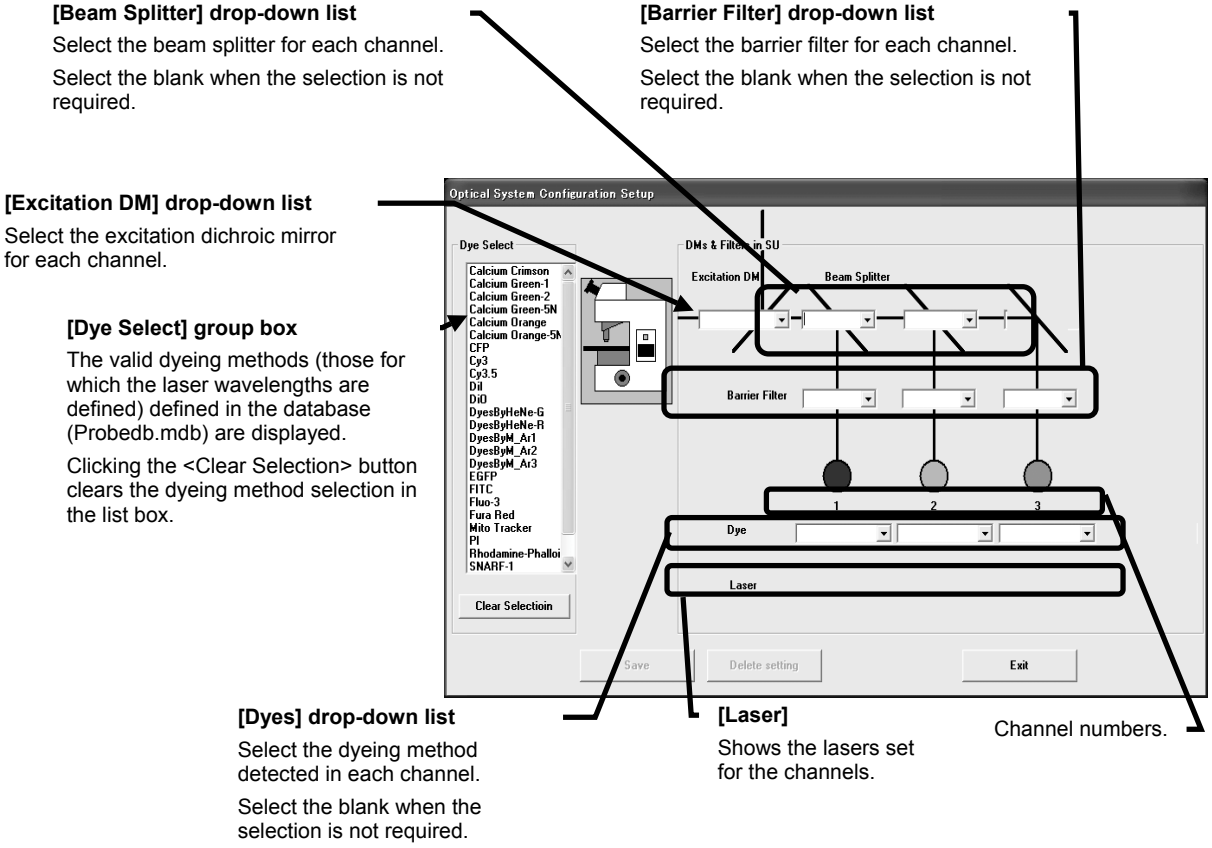
2. Click [Fluorescence] icon located at [User Settings] in [FLUOVIEW Setup] dialog and bring [Fluorescent Dyes/Colors] panel in front.



[Edit Dye Assign Database] button.

3. Click [Edit Dye Assign Database] button.

   [Optical System Configuration Setup] dialog box appears as shown below.

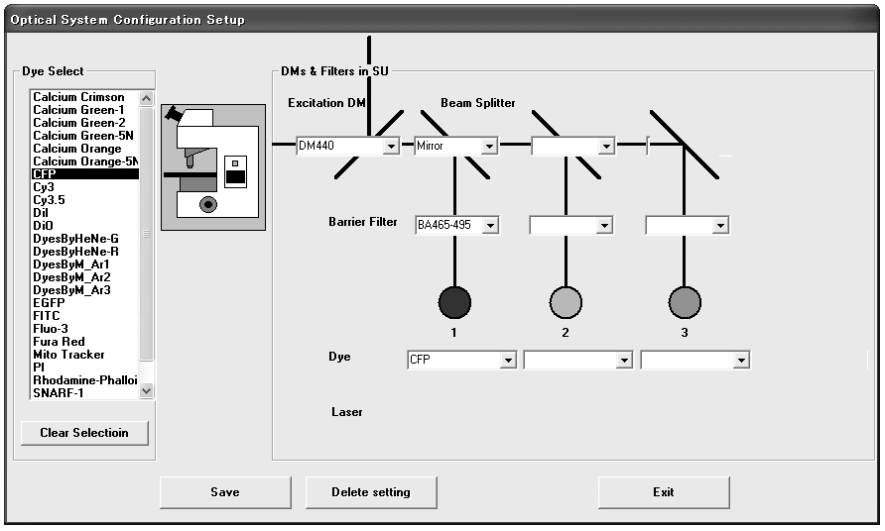

[Beam Splitter] drop-down list
Select the beam splitter for each channel.
Select the blank when the selection is not required.
[Barrier Filter] drop-down list
Select the barrier filter for each channel.
Select the blank when the selection is not required.
[Excitation DM] drop-down list
Select the excitation dichroic mirror for each channel.
[Dye Select] group box
The valid dyeing methods (those for which the laser wavelengths are defined) defined in the database (Probedb.mdb) are displayed.
Clicking the <Clear Selection> button clears the dyeing method selection in the list box.
Optical System Configuration Setup
Dye Select
DMs & Filters in SU
Excitation DM
Beam Splitter
Barrier Filter
Dye
Laser
Clear Selectioin
Save
Delete setting
Exit
[Dyes] drop-down list
Select the dyeing method detected in each channel.
Select the blank when the selection is not required.
[Laser]
Shows the lasers set for the channels.
Channel numbers.

4. In the list box in the [Dye Select] group box, select the dyeing method to be changed or created (CFP in this example). When a dyeing method is selected and if there is information associated with the selected dyeing method, the [Excitation DM] drop-down list shows the excitation dichroic mirrors, the [Beam Splitter] drop-down list shows the beam splitters, the [Barrier Filter] drop-down list shows the barrier filters, the [Dye] drop-down list shows the dyeing methods and the [Laser] group box shows the set laser types, all on the per-channel basis.

TIP To select more than one dyeing method, select the required methods while holding the Ctrl key.

TIP

Associations between dyeing methods and filters are saved per dyeing method combination, and applied when the combination is selected in the [Dyes] sub-panel of the FLUOVIEW software.



5. To change the excitation dichroic mirror, use the [Excitation DM] drop-down list.
   When it is not required to specify excitation dichroic mirror, select the blank.
   In this example, select ”DM440”.

6. To change the beam splitter, use the [Beam Splitter] drop-down list.
   When it is not required to specify a beam splitter mirror, select the blank.
   In this example, select “Mirror” for Channel 1.

7. To change the barrier filter, use the [Barrier Filter] drop-down list.
   When it is not required to specify a barrier filter, select the blank.
   In this example, select “BA465-495” for channel 1.

8. To change the dyeing method detection channel, set the [Dye] drop-down list of the previous channel to blank and select the dyeing method in the [Dye] drop-down list for the new channel.When it is not required to specify the detection channel, select the blank.
   In this example, select CFP for channel 1.

9. After completing the above, click the <Save> button to close the [Optical System Configuration Setup] dialog box.

TIP With the FLUOVIEW software, setting a filter to blank does not change the corresponding filter. Set blank only when any filter setting is acceptable.

2 Maintenance of Major System Units

This section describes the maintenance of the Microscope.

2-1 Laser Scanning Microscope

According to IEC60825 “Safety of Laser Product” and IEC60825(EN60825), this product is a CLASS 3B laser product. Therefore, all of activities such as attachment or removal of the microscope modules listed below are defined as the service activities.

I In the United States of America, this product is classified as a CLASS Ⅲb laser product according to the CDRH laser safety regulation, and attachment or removal of the microscope modules listed below is not permitted as part of user maintenance activities. (Such attachment and removal should be performed as part of service activities.)

For detailed imformation, refer to our service representatives.

1) Transmitted lamp housing
2) Transmitted light fiber cable
3) Cube turret of reflected light fluorescence unit
4) Objective revolving nosepiece
5) Objectives
6) Condenser
7) Differential interference slider
8) Excitation filter, spectral filter and barrier filter(Inside the scan unit)

The definitions of the term “maintenance” by IEC(EN60825) are quoted below for reference.

EN60825
Maintenance:
The performance of those adjustments or procedures specified in user information provided by the manufacturer with the laser product, which are to be performed by the user for the purpose of assuring the intended performance of the product. It does not include operation or service.

The definitions of the term “maintenance” by CDRH 21CFR are quoted below for reference.

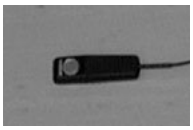
CDRH

“Maintenance” means performance of those adjustments or procedures specified in user information provided by the manufacturer with the laser product which are to be performed by the user for the purpose of assuring the intended performance of the product. It does not include operation or service as defined in paragraph (b) (27) and (38) of this section.

2-2 UV-Ar Laser

2-2-1 Auto Alignment Operation (Remote Switch)

The UV Ar laser is provided with an auto alignment facility, which automatically optimizes the laser oscillation by adjusting the unstability in output due to mirror alignment error resulting from long period of use. It is recommended to perform auto alignment about once a month.



Remote Switch

After warming up the laser head for more than 30 minutes, press the remote switch connected to the rear of the laser head once. The auto alignment starts and completes in some tens of seconds. The indicator on the rear of the laser head blinks during auto alignment.

Do not press the remote switch while the indicator is blinking.

Do not use the remote switch too frequently. The recommend interval is once every few months provided that the UV Ar laser is used frequently. When the UV Ar laser has not been used for a long period, press it only once at the time of idle running of the laser head (aging) which should be performed every month.

2-2-2 Checking, Refilling and Replacing Water in Chiller

1 Checking

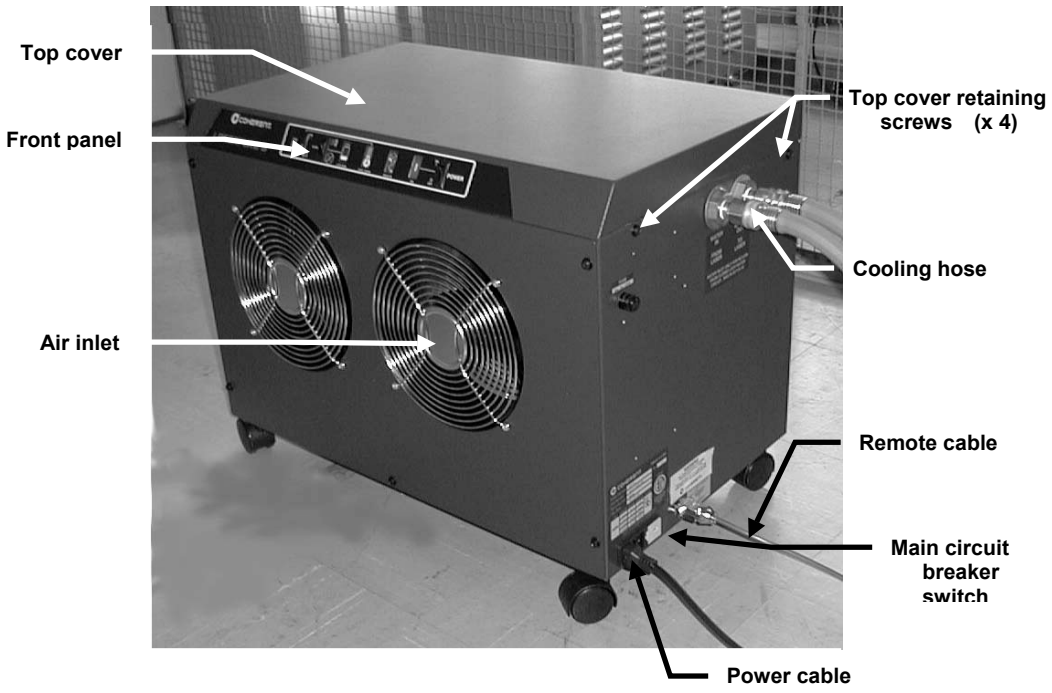
Chiller is a water-cooled system.

After long hours of use, check the water level and, if water is insufficient, **refill distilled water to the specified level.**

If the water in the tank is polluted, change the distilled water.

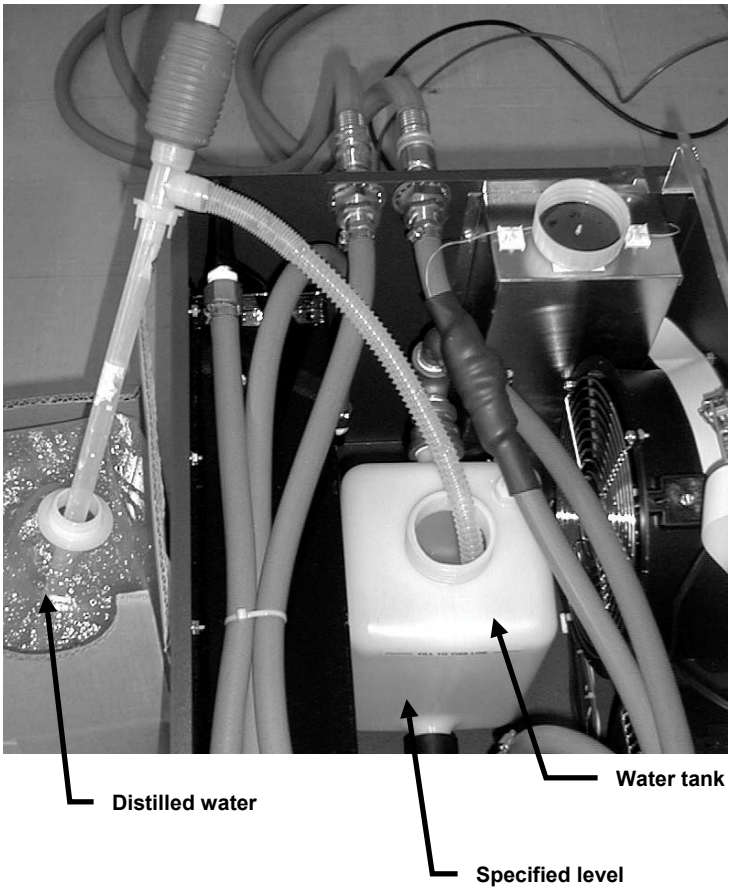
The check period is about once every six months. Note that the water tends to be polluted when the weather is warm or when the heat exchanger has been left unused for a long period.

1. Switch off the main circuit breaker of the heat exchanger and unplug its power cable from the power outlet.



2. Remove the 4 top cover retaining screws and remove the top cover.

3. Remove the water tank cover and check the level and pollution of water in it.



2 Refill and Replacement

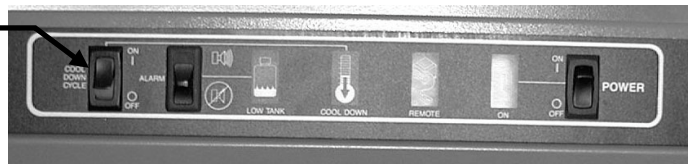
If the water level in the water tank is below the specified level, refill distilled water until the specified level.

If the water is polluted by algae or moth, replace the water to ensure cooling.

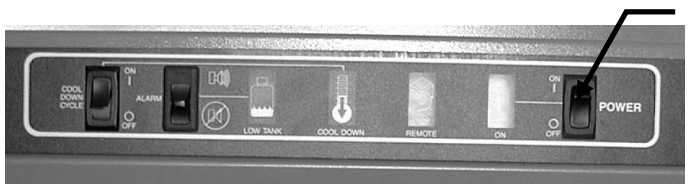
Replacement procedure

1. Set [COOL DOWN CYCLE] to “O-OFF”.

COOL DOWN CYCLE
Set to “O-OFF”.



2. Pump water out.
3. To eliminate water remaining in the cooling hose, switch [POWER] of the heat exchanger “I-ON”, run it idle and pump out water which comes inside the tank.



4. Refill new distilled water until the specified level. Run the heat exchanger manually and check the water level again. If it is below the specified level, refill additional distilled water.
5. Close the water tank with the lid, place the top cover and tighten the 4 retaining screws tightly using a screwdriver.
6. Plug the power cord into the power outlet and set the main circuit breaker to ON.
7. Set up for normal operation.
   For details, see section 1-2-1, “Turning Power ON” in Volume [OPERATION INSTRUCTIONS].

Consumable supply: Distilled water, about 10 liters (commercially available in 20-liter bottles).

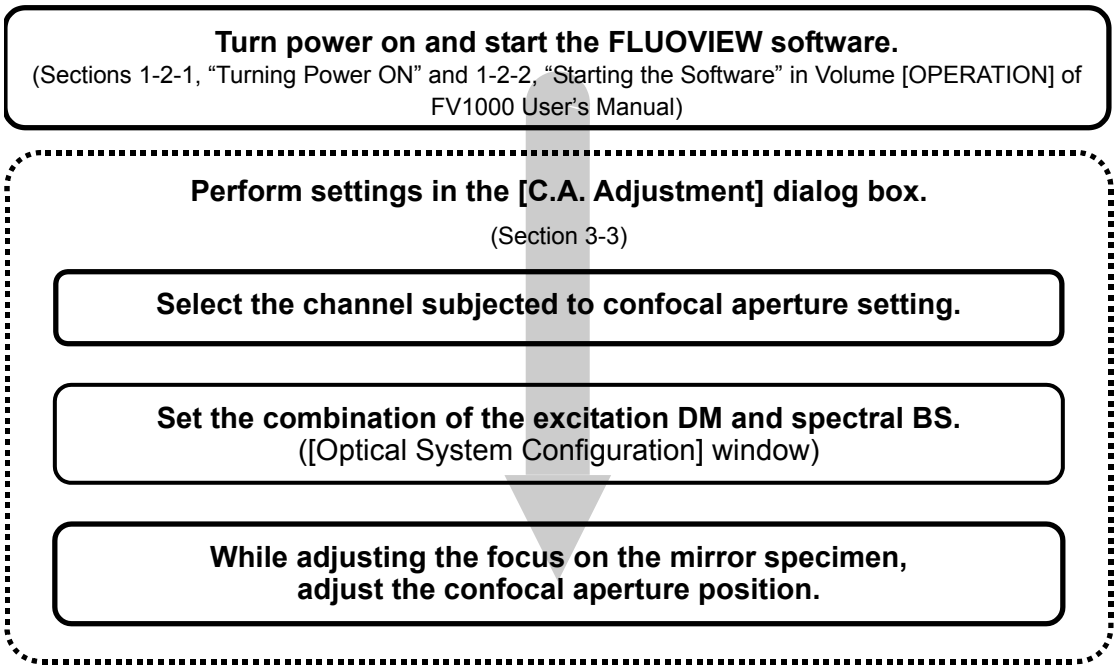
3 Setting the Confocal Aperture

The confocal aperture position is determined by the combination of the excitation dichroic mirror (hereinafter “excitation DM”) and spectral beam splitter (hereinafter “spectral BS”). If the confocal aperture position has not be factory set for the combination of excitation DM and spectral BS that you selected, it may become impossible to acquire correct images.

This chapter deals with how to adjust the confocal aperture position.

Follow the following.

For the procedure of each operation, refer to the section or manual indicated inside parentheses ( ).


Turn power on and start the FLUOVIEW software.
(Sections 1-2-1, “Turning Power ON” and 1-2-2, “Starting the Software” in Volume [OPERATION] of FV1000 User’s Manual)
Perform settings in the [C.A. Adjustment] dialog box.
(Section 3-3)
Select the channel subjected to confocal aperture setting.
Set the combination of the excitation DM and spectral BS.
([Optical System Configuration] window)
While adjusting the focus on the mirror specimen, adjust the confocal aperture position.

3-1 Starting the FLUOVIEW Software

Start the FLUOVIEW software.

For details, refer to Sections 1-1, “Turning the power On” and 1-2, “Starting the Software” in Volume [PREPARATION For OPERATION] of [HARDWARE GUIDE] of the FV1000 User’s Manual.

NOTE

To enable saving the confocal aperture setting, log in the FLUOVIEW software as the Administrator.

If you do not log in in the qualification of the Administrator, you will not be able to save the confocal aperture settings.

NOTE

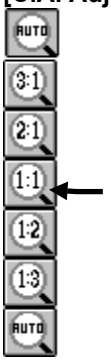
Image size of 512*512 will be selected in this procedure automatically.

Do not change the Image size during the procedure.

NOTE

Before starting image acquisition, select 1:1 magnification in the tool menu displayed on the top of [Live] panel.

If other magnification is selected, the adjustments in the [C.A. Adjustment] group box may become invalid.



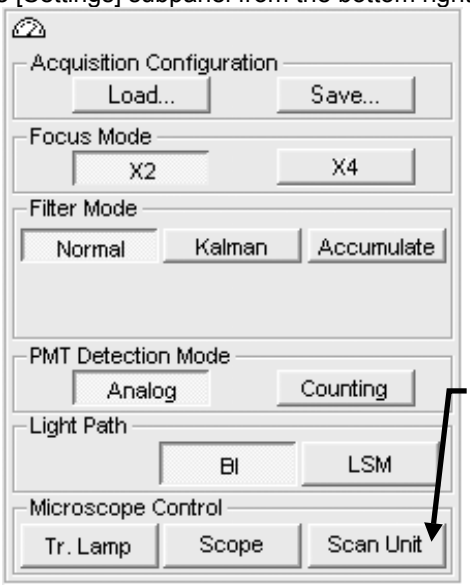
<1:1> button

See section 2-5-9, "Magnifying/Reducing an Image" in Volume [OPERATION] of FV500 User’s Manual for detail of the operation.

3-2 Displaying the [C.A. Adjustment] Dialog Box

Open the [C.A. Adjustment] dialog box of the FLUOVIEW software.

1. Select the tab for the [Settings] subpanel from the bottom right of the [Acquire] panel.



<Scan Unit> button
Click to display the types of the filters and laser used in setting.
(When the laser combiner is used, the laser is selected automatically).

Fig. 3-1 [Settings] Subpanel

2. Click the <Scan Unit> button.

   A window as shown below opens.

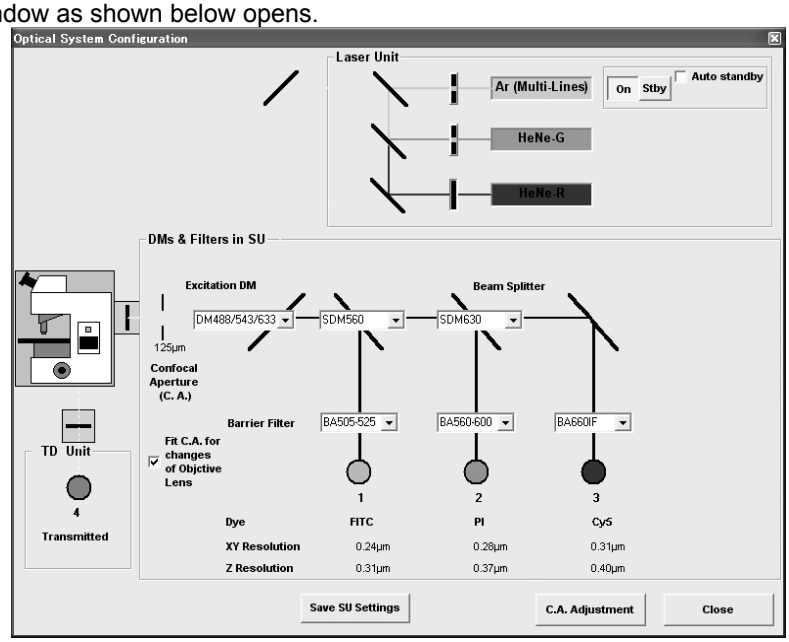


Fig. 3-2 [Optical System Configuration] Window

3. Click the <C.A. Adjustment> button.

   A dialog box as shown below opens.

Adjust the confocal aperture position.

The horizontal arrow moves it in the X-axis direction and vertical arrow moves it in the Y-axis direction.

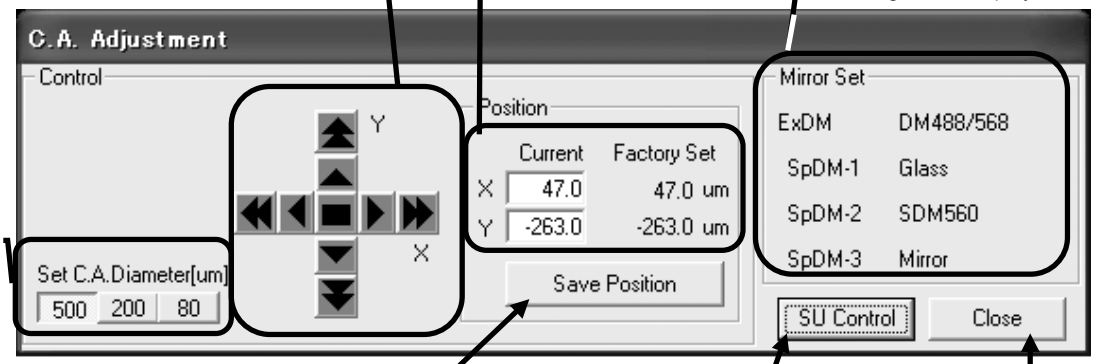
Each click of the inner button moves it by 8 µm and each click of the outer button moves it by 40 µm.

Click the center button to move it to the saved position.

[Current] text box
Shows the current confocal aperture position. This can be moved by entering a value directly in the text box using the keyboard.

[Factory Set]
Shows the factory-set confocal aperture position.

Shows the scan unit's internal settings when the [C.A. Adjustment] dialog box is displayed.



[Set C.A. Diameter] group box
Select the confocal aperture diameter.

<Save Position> button
Click to save the confocal aperture position in the position indicated by the [Current] text box .(Displayed only when the user logs in the FLUOVIEW software as the Administrator.)

<SU Control> button
Click to activate the [Optical System Configuration] window.

<Close> button
Click to close the [C.A. Adjustment] dialog box.

NOTE

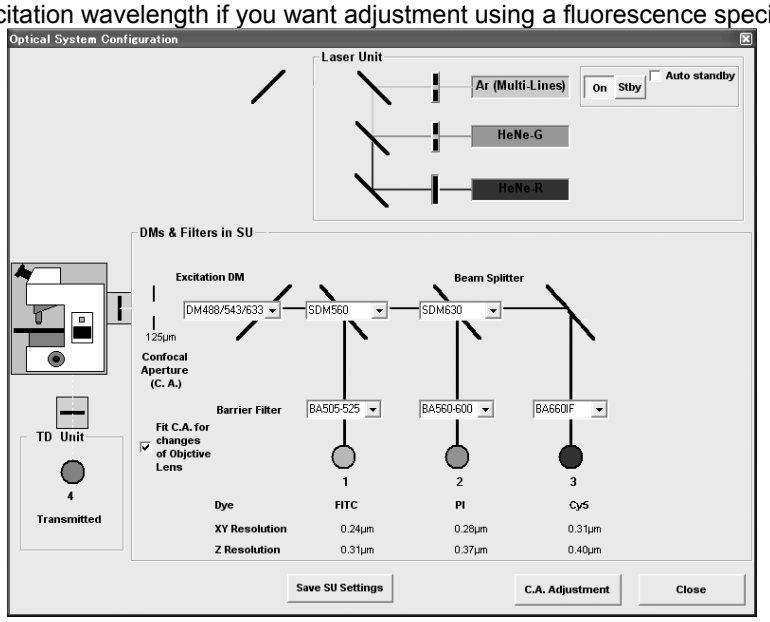
If the message below appears by clicking the <C.A. Adjustment> button, exit from the FLUOVIEW software and execute "FVCAReader.exe." in the "FLUOVIEW" folder under drive C.

Project1
PinholeDefault.ini file is not found.
If you want to adjust the pinhole position, exit from FLUOVIEW and execute FVCAReader.exe file.
OK

3-3 Confocal Aperture Setting

1. Engage the 10X or 20X objective in the light path and place the mirror specimen on the stage. (Place a fluorescence specimen if you want to perform adjustment using a fluorescence specimen.)

2. In the [Laser Unit] group box in the [Optical System Configuration] window, select the wavelength of the laser used in confocal aperture setting (488 nm). (Select the excitation wavelength if you want adjustment using a fluorescence specimen.)



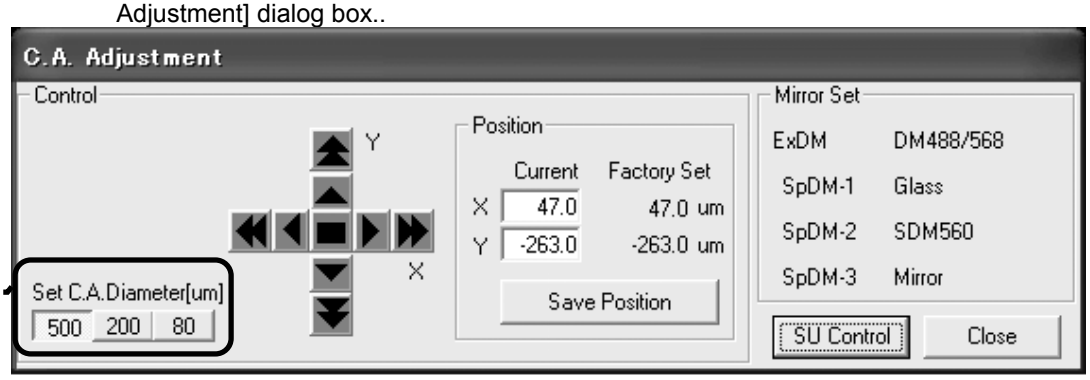
3. Click the <SU Control> button. When the [Optical System Configuration] window is displayed, set the excitation DM and the spectral BS of the channel you want to set the confocal aperture. (For the display, see step 2.)

NOTE

Set the spectral BSs of the channels closer to the excitation DM than the adjusted channel (on the left side of the adjusted channel in the screen) to "Glass". As the channel shown at the rightmost position in the [DMs & Filters] checkbox in the [Optical System Configuration] window does not have the spectral BS, it is simply required to set the spectral BSs of all channels to "Glass" when adjusting the confocal aperture of the rightmost channel.
This makes it possible to increase the detection light intensity.

4. In the [DMs & Filters in SU] group box in the [Optical System Configuration] window, set the barrier filter of the channel you want to set the confocal aperture to "Empty. (If you want adjustment using a fluorescence specimen, cut the excitation wavelength and set the barrier filter for absorbing the fluorescence.)
(For the display, see step 2.)

5. Click the <500> button in the [Set C.A. Diameter] group box in the [C.A. Adjustment] dialog box..

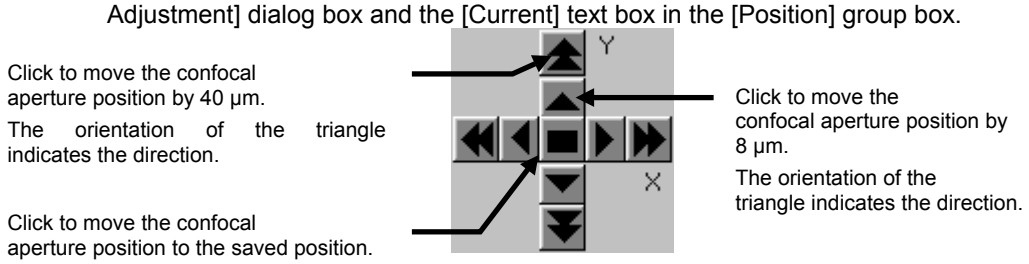


[Set C.A. Diameter] group box
Select the confocal aperture diameter.

6. Click the <XY Repeat> button on the upper part of the [Acquire] panel to started repeated image scanning.

7. Rotate the focus adjustment knob of the microscope to bring the mirror surface into focus. The focal position is the position where the image is bright and displays scratches on the mirror surface most clearly.

8. While checking the repeated scanning image on the image window, adjust the confocal aperture to the position that provides the brightest image by using the arrow buttons on the center of the [Control] group box in the [C.A. Adjustment] dialog box and the [Current] text box in the [Position] group box.


Click to move the confocal aperture position by 40 µm.
The orientation of the triangle indicates the direction.
Click to move the confocal aperture position to the saved position.
Y
X
Click to move the confocal aperture position by 8 µm.
The orientation of the triangle indicates the direction.

TIP The confocal aperture position can be moved between –750 and 750 µm.

9. Click the <200> button in the [Set C.A. Diameter] group box.
   (For the display, see step 6.)

10. Rotate the focus adjustment knob of the microscope to bring the mirror surface into focus. The focal position is the position where the image is bright and displays scratches on the mirror surface most clearly.

11. Perform the confocal aperture adjustment in step 8 so that the image is brightest.

12. Click the <80> button in the [Set C.A. Diameter] group box.
    (For the display, see step 6.)

13. Rotate the focus adjustment knob of the microscope to bring the mirror surface into focus. The focal position is the position where the image is bright and displays scratches on the mirror surface most clearly.

14. Perform the confocal aperture adjustment in step 8.
    The correct confocal aperture position is the position with which the bright part comes on the center of the screen.
    The bright part may sometimes move across the stop edges of the image window during the focal position adjustment. Adjust the focus and confocal aperture position alternate for a few cycles.

15. Click the <Save Position> button to save the confocal aperture position.

TIP

The saved position becomes the initial setting for the confocal aperture at the moment the FLUOVIEW software is started. This position is set from immediately after the save operation.

16. Click the <STOP SCAN> button in the [Acquire] panel to stop image scanning.

17. Adjust the confocal aperture positions of other combinations of excitation DM and spectral BS, which have not been set yet, by repeating steps 1. to 16.

18. Click the <Close> button to close the [C.A. Adjustment] dialog box.

TROUBLESHOOTING

On This Volume

This volume describes the treatment against possible troubles.

In case of trouble, please read volume before calling for service. If the normal operation cannot still be restored, please contact your local Olympus representative.

1 TROUBLESHOOTING GUIDE 1-1

1. Message – “Scanning Unit hardware not responding. Acquisition Not Enabled” appears. 1-1
2. Message – “Microscope hardware not responding. Acquisition Not Enabled” appears. 1-1
3. Message – “Scan hardware not responding. Initializing as FLUOVIEW Review Station” appears (In case that Review Station is used). . 1-1
4. The only first image appears in the Live view. 1-1
5. Message – “Unable to complete scan...” appears during scanning. 1-1
6. Message – “WaitForMalStatusFlag(): TimeOut failure. Execution will continue” appears. 1-1
7. Image file can not open with ? marked in the file list. 1-1
8. The comment color is not same with its assigned. 1-1
9. FLUOVIEW software cannot be booted up. 1-2

1 TROUBLESHOOTING GUIDE

Under certain conditions, performance of this system may be adversely affected by factors other than defects. If a problem occurs, please review the following list and take remedial action as needed.

If you cannot solve the problem after checking the entire list, please contact your local Olympus representative for assistance.

Before contacting Olympus, please fill the “Inquiry Table” at the end of this volume and inform Olympus of its contents.

Phenomenon	Cause	Treatment	Manual Ref. Pages
1. Message – “Scanning Unit hardware not responding. Acquisition Not Enabled” appears.	Power of power supply unit (F10-PSU) is not turned on.	Turn power of power supply unit (FV10-PSU) to ON position.	-
2. Message – “Microscope hardware not responding. Acquisition Not Enabled” appears.	Power of control box (UCB) is not turned on.	Turn power of control box (UCB) to ON position.	-
3. Message – “Scan hardware not responding. Initializing as FLUOVIEW Review Station” appears (In case that Review Station is used).	Setup contents are not correct.	Verify if setup contents are set to Review Station.	“Maintenance Section 1-3-1”
4. The only first image appears in the Live view.	Previous Live View window is locked.	Set the channel to display in the window after the scanning.	“New Function Section 1-2-3-3” “OPERATION INSTRUCTIONS Section 2-5-4-2”
5. Message – “Unable to complete scan...” appears during scanning.	Program becomes busy.	Terminate unnecessary programs.	-
6. Message – “WaitForMalStatusFlag(): TimeOut failure. Execution will continue” appears.	Something wrong with the hardware connection.	Confirm the connection of the cable with shutting the system down. Then, reboot the system.	“PREPARATION For OPERATION Section 1”
7. Image file can not open with ? marked in the file list.	The name is over 120 characters include the path.	Move the file to the other folder to shorten the file name which includes the path, with in 120 characters.	-
8. The comment color is not same with its assigned.	Overlay coloring is set to change sequentially.	Change the setting with FV setup.	Step 8 Of “Maintenance Section 1-3-1”

Phenomenon	Cause	Treatment	Manual Ref. Pages
9. FLUOVIEW software cannot be booted up.	Something wrong with the 'ini' file.	1.Terminate FLUOVIEW software and FLUOVIEW Setup. 2.Delete [GBSTATE.ini] file in [FLUOVIEW\System] folder in HDD where FLUOVIEW soft ware is installed, Then set it up again by FLUOVIEW Setup program. 3.Boot up FLUOVIEW software.	"Maintenance Section 1-3"

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